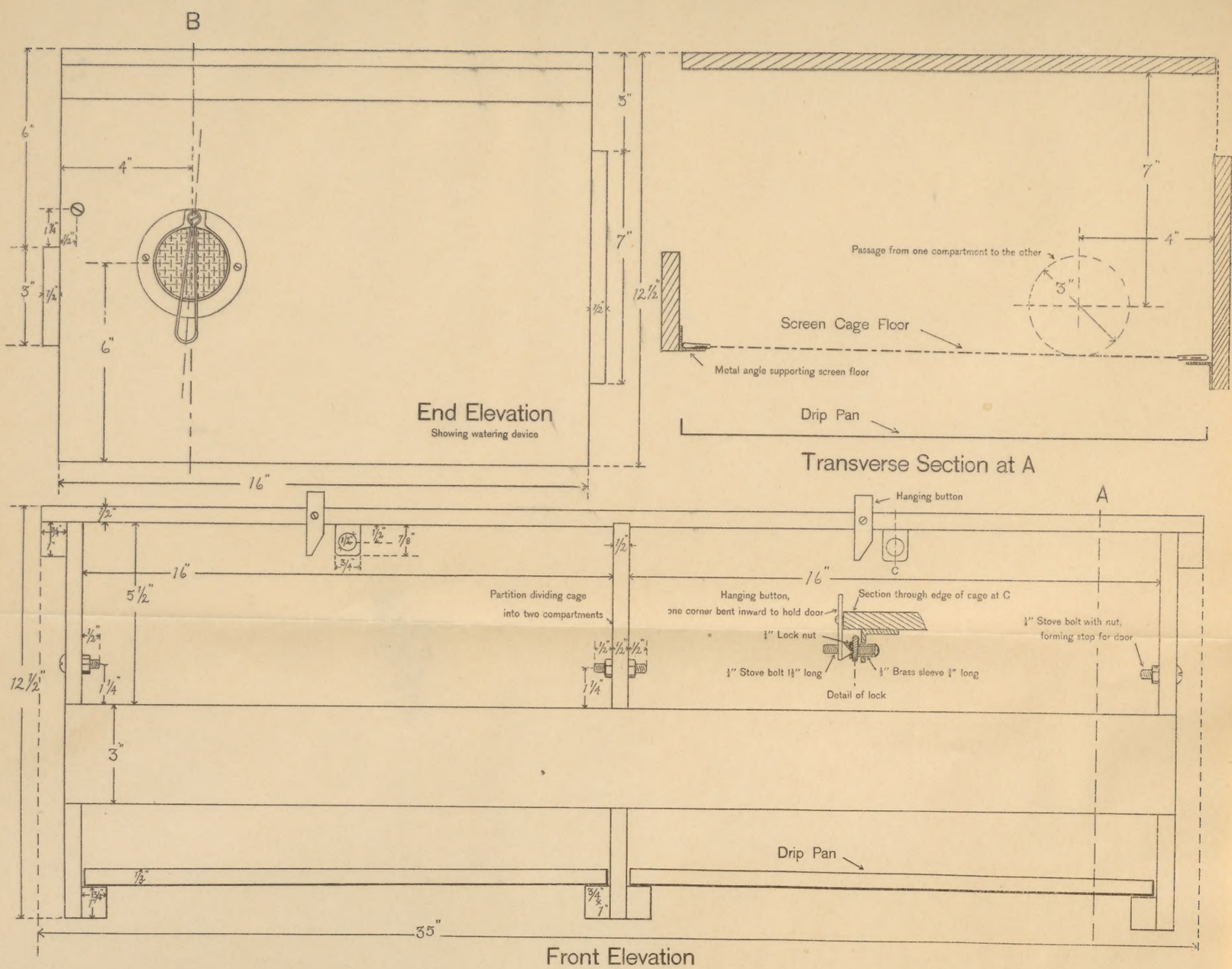
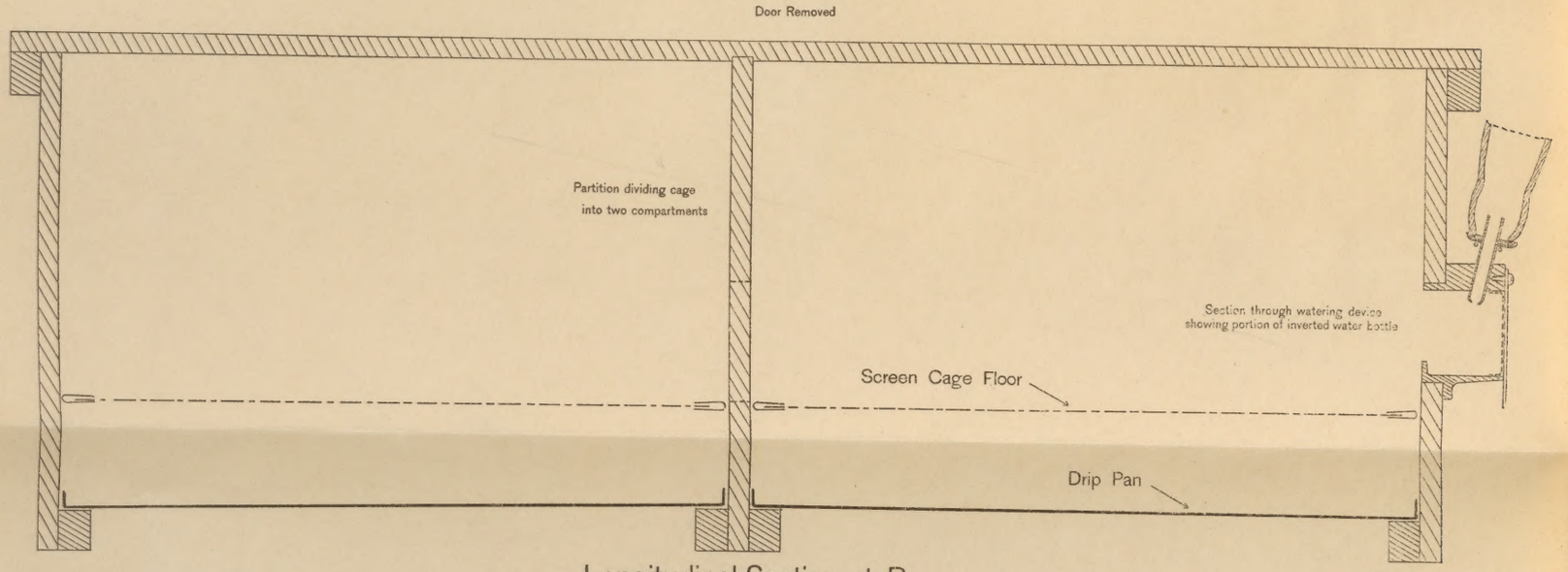
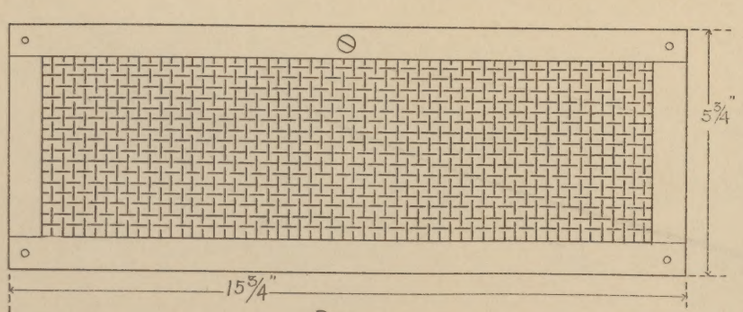
BREEDING AND CARE OF THE ALBINO RAT FOR RESEARCH PURPOSES

MILTON J. GREENMAN
AND
F. LOUISE DUHRING



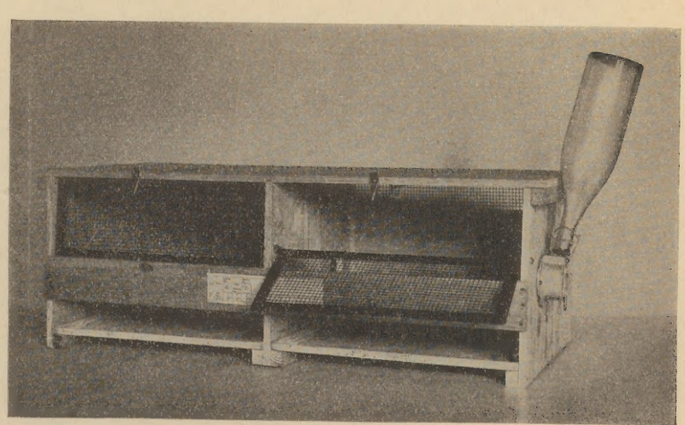


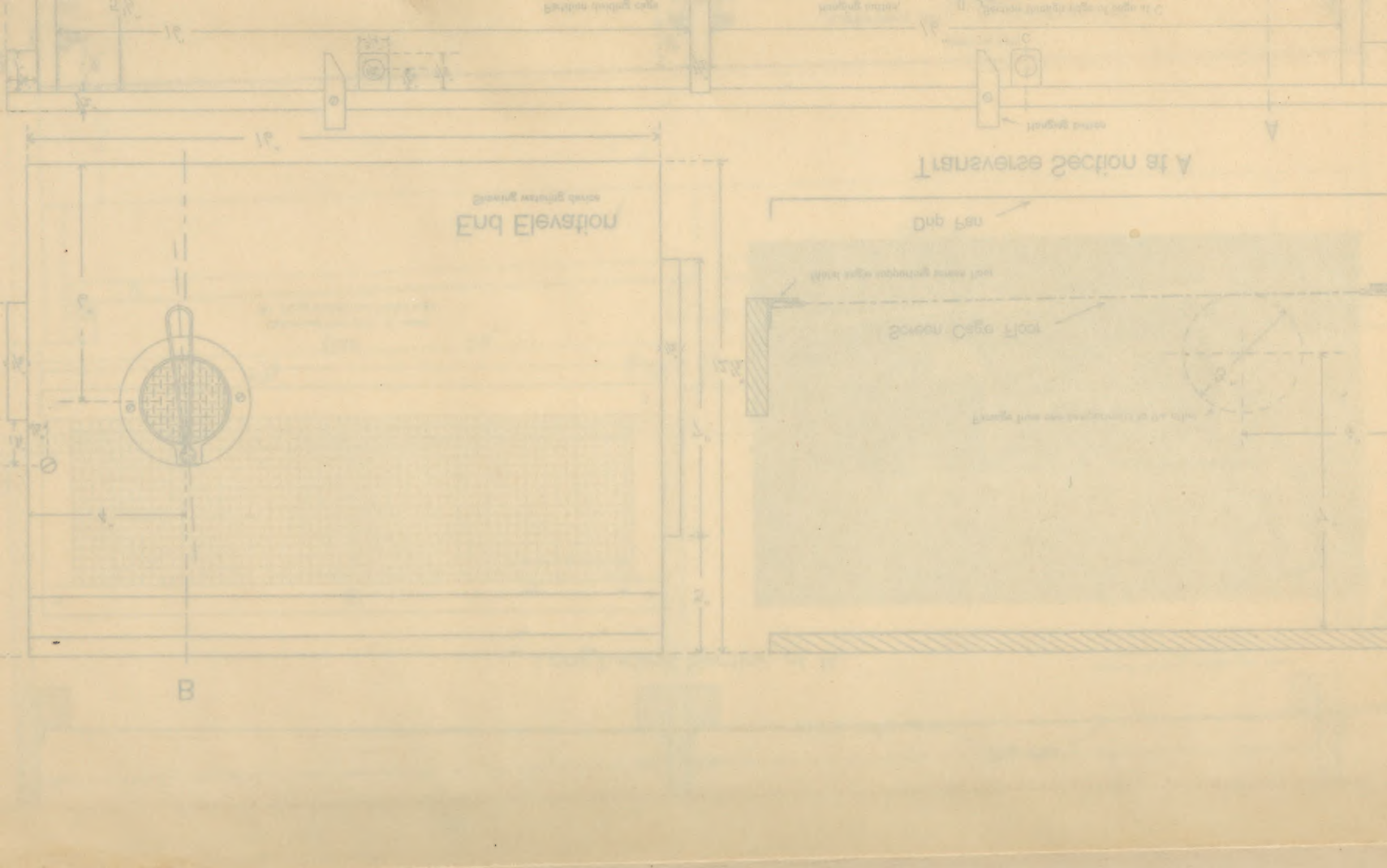
Longitudinal Section at B



Door

Galvanized wire cloth, ¼" mesh
No. 22 galvanized iron folded edge

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PREFACE

The preparation of this little book was suggested by the numerous inquiries which we are constantly receiving regarding the methods followed in breeding albino rats.

During the past sixteen years The Wistar Institute has maintained a colony of albino rats to provide the necessary material for the investigations carried on in its laboratories.

Beginning with a small number, this colony grew to very considerable proportions. During its time the colony has passed through all the various forms of colony care, management, and equipment that were suggested as remedial for its numerous difficulties.

It became more and more evident that clean, healthy, albino rats were essential for accurate research and that their production was a serious, difficult, and worth-while task.

By a more intimate acquaintance with the habits of this little animal and a study of the means of making it contented and happy, apparatus and methods were devised for developing a colony which, while far from perfect, is very much better than the colony of the older methods.

It was necessary to devise new cages, new diets, new methods of handling albino rats, and finally a new building where the various reforms of colony management could be carried out more successfully and more economically.

The authors have attempted to put into form for the use of albino rat breeders, technicians, and others who are charged with the care of albino rats, such information as they have gained from a long and varied experience in breeding this mammal.

During the experimental work with the colony a considerable amount of new information regarding the natural history of the albino rat was recorded and remains yet to be pub-

CONTENTS

ILLUSTRATIONS

CHARTS

Anatomical and physiological investigations of an experimental kind have become more or less quantitative in character. To insure more accurate results in such investigations it has been necessary to select, as an experimental animal, a type which, under uniform environmental, nutritive, and psychological conditions, may be expected to follow with a minimum of variation certain predetermined standards of behavior during its entire span of life. This is only possible in a relative degree.

The animal chosen for this purpose should be of the greatest possible uniformity in vitality, weight, structure of its organs and parts, at given ages, and in transmitting power—a stock as nearly homozygous as possible. In other words, a standardized animal is needed for the most accurate results in biological research.

On account of its numerous virtues as a laboratory animal, the albino rat (Mus norvegicus albinus) has come into favor for research purposes.

It is easily kept in fairly small quarters; lives and grows on almost any food utilized for human consumption. It is not expensive to maintain when the value of a standard animal is considered. Its omniverous habits make it an exceptionally valuable animal for nutritional investigations, since the effects of an exclusively vegetable diet or those of a strictly animal diet may be observed. Its span of life is short, about three years; the female mates when about eighty days of age, the gestation period is from twenty-one to twenty-three days, and it may mate within two days after casting a litter. It is prolific—a female may produce nearly a hundred pups in a year—and it is possible to secure three or four generations in a year. The young are very immature at birth, which permits

of experimental modification of cells and the growth of organs. Owing to the large number in each litter, from six to twelve, it is especially valuable for experimental purposes, since control and experimental animals may be selected from the same litter. Animals from the same litter are more nearly alike than others of the same age.

The recent work of Long and Evans has given us a method of determining with accuracy the condition of the reproductive tract through the several stages of the rat's oestrous cycle. This, together with the large number of embryos produced at one time and the ready means of selecting females which have just been inseminated, gives the rat special qualifications for the embryologist.

As a domesticated animal which may be readily gentled, it is responsive to care and attention and thrives under conditions of comfort and contentment. It soon becomes accustomed to, or even develops an interest in, activities about it, evinces an affection for its caretakers, and shows its pleasure in playful pranks not unlike other domestic animals. All these add value to it as a laboratory animal, since it makes training easy, affording opportunity for behavioristic observations such as the study of function and growth when they are induced to take exercise in a revolving cage. The albino rat may be gentled so that laboratory handling will produce a minimum of disturbance of the normal physiological processes which may be under observation.

As a mutant from the wild Norway rat (Mus norvegicus) we have a domesticated form to compare with its wild ancestor. The effects of domestication may be observed and measured. Both forms are cosmopolitan so that the results of research on this mammal are comparable though done in widely separated localities.

As pointed out by McCoy (The Rat and its Relation to the Public Health, by various authors; P. H. and M. H. Service Bulletin no. 30, Washington, 1910), in discussing "Organic Diseases of the Rat, Including Tumors," the wild gray rat

suffers spontaneously, although rarely from cirrhosis of the liver, fatty degeneration of the liver, nephritis and calculi of the urinary tract, and for this reason would probably furnish excellent subjects for experimental investigation of these diseases.

While the albino rat is small, requiring a more delicate technique, it is serviceable for many operative procedures, and its unusual immunity to surgical infections qualifies it especially for such experiments.

There are now available more records on the growth of the albino rat and its organs and parts than exist for any other mammal. This has resulted largely through the efforts of Prof. H. H. Donaldson and his co-workers who have used the albino rat in a study of the growth of the nervous system. Early in these investigations Professor Donaldson recognized the significance of age in the study of structure and the value of determining a factor by which experimental results might be applied to man.

A rat of three years is equivalent in age to a man of ninety years. Both are at the close of the life-span. The rate of growth in the rat is thus thirty times as rapid as in man. Development in the two forms is in the same stage when equal fractions of their life-spans are compared.

Thus it is possible to verify or apply directly to man experimental data obtained on the albino rat. No other form is at present sufficiently well known to be utilized in this manner.

The desirable conditions enumerated in this chapter are those brought to our attention by many years of experience.

In the new colony building of The Wistar Institute, completed in 1922, practically all the features here presented have been incorporated.

The need for ideal conditions increases geometrically with the increasing size of the colony and the more exacting requirements of the investigator.

Colony rooms should not be located in the dark, dank spaces of a basement nor in the cramped, hot areas of an attic.

No space unfit for human habitation is desirable for an albino rat colony. The ideal colony building or room should be abundantly supplied with direct sunlight and ample provsion should be made for rapid change of air. If possible, areas connected with the rooms should be provided where rats may be exposed to direct sunlight, unfiltered through glass windows.

The work of a colony is greatly facilitated if the entire colony is located on one floor level, preferably the ground floor.

There should be protection against excessive heat in summer, by attic space or rooms above the colony rooms.

Heat should be regulated in cold weather so that extremes of temperature may be avoided, especially the sudden drop in temperature. From 65° to 75°F. would seem to be a very satisfactory range of temperature.

Unfortunately, it is not always possible to avoid the higher temperatures of summer. Some compensation may be made, however, by rapid change of air in the colony rooms and thus remove some of the heat and moisture generated by the rats themselves. Forced ventilation is always more satisfactory because it may be regulated and controlled.

Colony rooms must be protected against wild mice and wild rats which may bring parasites and diseases.

All openings should be carefully screened to exclude flies which may carry infections and will breed in the cages.

Building construction is sometimes fixed and beyond control. The ideal building for an albino rat colony should be constructed of brick, stone, concrete, steel, and glass. These materials offer less harbor for dirt and vermin if properly put together. Floors should be tight and smooth, preferably of tile, concrete or magnesite, so that they may be washed. Magnesite floors may be laid over wood.

Rooms should be amply supplied with water outlets. Electricity is almost a necessity, both for light and power.

Where a number of rooms are used for a colony, it is desirable to have an outside door for each room. If a room becomes infected it may then be sealed off from the remaining rooms of the colony.

The cage equipment may be hung from the ceiling with decided advantages. Each bank of cages in a room is thus isolated from the others. The floor is then free and easily cleaned and affords no hiding places for escaped rats. A small colony laboratory is desirable not only for its convenience to the colony, but also to avoid the necessity of operations in the colony and to spare the rats the annoyance and ill effects of laboratory odors.

A cleaning and sterilizing room is an essential where all work of cleaning cages may be done. The steam and noise of such work react unfavorably upon the rats. Such a room should be provided with a tank for washing and boiling cages, a sterilizer for sterilizing bedding and all articles that are brought into the colony.

The large steam sterilizers are, of course, the best. The ideal method is to locate the sterilizer, having a door at each end, in a partition wall, on one side of which all materials for the colony which may be infected are received and placed in the sterilizer and on the opposite side they are removed sterilized ready for use in the colony.

A kitchen should be provided where food may be prepared without the disturbing effects on the colony. While it is possible to maintain a colony without cooking food we have preferred to sterilize by cooking all foods that might possibly carry parasites. The cooking process may also be utilized to make more palatable food combinations. A steam soup kettle or a range will be found useful. Pots, pans, pails, dippers, large spoons, a cleaver, a butcher's saw, meat block, table and a large sink with hot and cold water, will be necessary. A hand or power meat grinder is a useful part of the kitchen equipment. We have found a steam drying apparatus a time-saving and very useful device for desiccating certain cooked foods which are to be kept some time before feeding. It serves also to dry in minutes of time the pans, feeding dishes, cage floors, cages, etc., which usually take hours by any other process. Food trucks with trays for distributing food are essential in a large colony. Large fiber boxes are best for collecting the waste and dirt from the rooms. Metal cans are too noisy.

In a large colony it is desirable to have a record room or office where records and all information regarding the colony may be found. These records should indicate the various strains of rats on hand, their ages, number, location and any other information of importance. It is undesirable to permit comparative strangers to look through the cages for the particular animals they may wish for their research work. Suitable records in the office will make such visits unnecessary. The methods we follow are described elsewhere.

Finally, a store room should be conveniently located near the colony where all supplies may be kept. Such a store room should be protected from vermin.

CAGES

In the selection of cages one will be guided by the type of investigations for which albino rats are to be used and the length of time such investigations are to cover. The conditions necessary for rearing standard rats in considerable numbers are much more elaborate than those for short-time experiments with animals supplied at the proper ages or weights.

In considering the subject of cages, we are presenting the results of our experience in breeding and rearing albino rats in large numbers.

Confining a rat to the limited quarters of a cage necessarily restricts its activities, modifies its mental processes, and influences its growth and development. During the process of domestication the albino rat has undergone distinct changes in its general anatomy, with a considerable reduction in its body weight when compared with its wild ancestor. The changes in its anatomy with a reducton of body weight may have been one of the results of inadequate quarters, unsuitable food, and improper care in the hands of many breeders during all the years of domestication. Be that as it may, the albino rat has not only become accustomed to, but even dependent upon such limited abode as is usually furnished for shelter and protection.

To develop good, vigorous albino rats, they should not only be fed and sheltered, but compensation should be made, as far as is possible, for the disadvantages of cage life.

Recent experiments lead us to believe that fear and lack of exercise are factors which react unfavorably upon the growing rat. Cages may be so constructed as to aid in eliminating these factors to some degree. Within reasonable limits, the larger the cage, the more satisfactory will it be.

The chief disadvantage of the large cage, however, especially one of considerable depth, is the opportunity it affords the rat of secreting itself and avoiding contact with the caretaker, which is so essential in eliminating fear.

The cage should be protected from excessive cold and excessive heat; from sudden changes in temperature and especially from dampness. The rat will live in wide ranges of temperature. It is possible that it may even become more vigorous in a temperature varying within reasonable limits—a point yet to be determined. For the successful operation of a colony, however, a temperature ranging between 65° and 75° F. is desirable not alone for the rats, but for the comfort of the curators and caretakers who can perform their duties in a more efficient manner in a comfortable temperature.

Excessive heat combined with excessive humidity is undesirable. A number of rats huddled together in a cage generate a large amount of heat and give off quantities of moisture. The temperature within an overcrowded and inadequately ventilated cage rises several degrees above the surrounding atmosphere. It is essential, therefore, to have ample ventilation for each cage without sacrificing other features.

In a well-ventilated cage it should be possible for the rat to protect itself from undesirable draft or enjoy the air when desired by the selection of a suitable sleeping spot and the proper arrangement of its bedding.

Light is essential for the health and happiness of the rat and hence its growth and fertility. Recent observations on the occurrence of rickets in human subjects reared in darkened rooms as well as experimental tests on albino rats indicate the necessity of light to obtain perfect metabolism.

The most perfect abode for animals should include an open space where they may come into direct sunlight which does not pass through glass or any medium which may filter out essential light rays.

A cage, however, should afford a dark space where the rats may seek shelter for their nests, where they may be protected

from the light or secrete themselves in case of fear, yet be within easy reach of caretakers.

The cage should be provided with a dry floor covered with bedding, such as clean straw, hay, or planer shavings. In addition, parchment-paper clippings or 'wood wool' or 'excelsior' should be supplied for nests in which the mother may care for her pups.

Our experience leads us to prefer pine planer shavings for the bedding of the cage and 'wood wool' for nesting materials. These materials act as absorbents, disinfectants, and deodorants. Parchment-paper clippings, which we have also used, are good, but have the disadvantage of adhering sometimes to the mouth and nostrils of new-born pups causing death from suffocation. In the cages here described we use the finest grade of 'wood wool' exclusively for bedding and nesting material. In it the rats may form burrows and build nests to gratify their own tastes.

The necessity of maintaining cleanliness in cages at once raises the question of materials from which cages shall be constructed. The collapsible metal cage is easily handled, cleaned, and sterilized. It folds into small space when not in use. It is more durable than wood.

The selection of materials for cages, however, will depend upon climate, the conditions of the experiment, the length of time rats are to be kept, and other conditions.

The wooden cage is preferred by the rat in Philadelphia. It offers some protection during a sudden fall in temperature. During cold weather the rat will build its nest in a wooden box instead of a metal one, if permitted to choose. Nests are frequently located so that the outer wall of the cage forms one side of the nest wall.

It has been our experience, after trying both wood and metal cages, that better results in a large colony may be had by the use of wooden cages, other factors being equal.

For the production of the best animals two types of cages are desirable. First, a dormer cage and, second, an exercis-

ing cage. A revolving cage or a turntable is used for the latter purpose.

The two forms of cages which we have adopted after considerable experimental work with several types of cages are here described.

Dormer cage

To distinguish them from other types of cages, like the exercising cage, the special cages used for experiments in nutrition, etc., we have designated as 'dormer cages' (dormir, to sleep) those cages in which stock animals are bred, reared, and housed.

The dormer cage is constructed of New England white pine ½″ thick, and is 35″ long, 12½″ high, and 17″ deep, all outside measurements. It is divided in the middle by a partition into two compartments, each compartment measuring 16″ x 16″ inside, with a height of 8½″ from the removable screen floor to the ceiling. The two compartments communicate through a circular opening 3″ in diameter, located near the rear of the cage. The object of this division is to increase the number of more or less sheltered positions where the rat may build its nest or protect itself from direct lights. It affords the rat an opportunity to escape from one compartment to the other if frightened. This simple shifting of location appears to satisfy the animal that it has protected itself. Furthermore, it is sometimes desirable to close the opening in the dividing partition in order to confine the rats in one compartment while the other is being cleaned.

The cleats used in the construction of the cage, across the ends, and on the lower edge of the dividing partition are all without the cage, thus avoiding projecting wood angles within the cage.

The circular opening from one compartment to the other presents the only angle or edge within the cage which the rat may gnaw. This edge is protected by a metal band.

Everywhere else within the cage the wood presents only flat, smooth surfaces which the ordinary albino rat will not attempt to gnaw. Special protection must be provided in cages which are intended to contain extracted albino rats.

For the cage floor in each compartment, a removable galvanized wire-cloth screen 15¾″ x 15¾″ (no. 22 wire ⅛″ mesh) having a folded edge ¾″ wide, of no. 22, galvanized sheet steel is provided. This screen floor is supported along the front and along the rear by ¾″ x ¾″ x 1/16″ galvanized steel angles. Wood or fiber floor may be used if desired for protection against cold. Beneath the removable floor is a galvanized sheet steel drip pan or tray, 15¾″ x 15¾″ x ½″ deep. This pan catches the drips and finer particles of dirt falling through the cage floor.

At the right-hand end of each cage is the drinking fountain carrying a one-quart water bottle. This drinking fountain consists of a galvanized iron pocket opening into the side of the cage. Into this pocket projects the metal tube outlet of the inverted water bottle. A fresh drop of water always hangs on the slightly constricted outlet of this tube. Here the rat may drink, but it cannot foul the water supply. Furthermore, the outer end of this iron pocket carries a wire screen to admit light. This inhibits the rat from filling the space with food, litter, or other materials, and thus interfering with the proper working of the drinking fountain. The drinking fountain is so constructed that all excess water dropping from the water bottle flows out the end of the cage, thus preventing the wetting of the cage and the bedding on the cage floor.

The two doors of the cage are made of galvanized wire cloth, ¼″ mesh, with a folded edge ¾″ wide of no. 22 galvanized sheet steel riveted at the corners. In the middle of one side a stove bolt with suitable brass sleeve and lock-nut furnishes both a handle for the door and a part of the locking mechanism. It is well to paint the wire-cloth door with some hard black enamel. This renders the contents of the cage more plainly visible.

The doors are not hinged to the cage, but set into the front openings of the cage, so as to protect all exposed edges of wood and so they may be easily removed. In feeding, the caretaker may unlock and open a cage door with one hand while serving food with the other.

At the rear of the cage and along its entire length at the upper part, a 2½″ opening covered with ¼″-mesh galvanized wire cloth affords ventilation for the rats.

The cage is so constructed that one may set upon the top of another in banks of four or five as may be desired. No projecting part interferes with this arrangement. Or they may be supported on a rack with projecting arms which would come just under the overhanging upper edges of the ends. With such a supporting rack, each cage may be removed without disturbing others.

Our own practice is to pile the cages five high on an angle-iron frame suspended from the ceiling so that the lowest cage is 14 inches from the floor.

The light weight of such cages makes it possible for a person with a small amount of physical strength to handle cages. Such a cage, stripped of its accessories, weighs 14½ pounds.

In cage construction the economy of operation should be considered if there are many cages in service. Light-weight cages can be cleaned and sterilized more quickly than heavy ones. The lighter the cage, the less heat units will be required to sterilize it.

The dormer cage here described is intended to accommodate one breeding pair of rats and a litter of young. Not more than eight or ten adult albino rats should be kept in a cage of this size.

The construction and dimensions of the dormer cage and its parts are shown in figure 1.

Exercising or revolving cage

The exercising cage is an essential part of the colony equipment if fertility is to be maintained and vigorous rats are desired.

The cage which we have found very satisfactory is constructed upon a 21-inch bicycle wheel. The excellent ball bearings of a bicycle wheel are essential, for revolving cages are subjected to a very considerable daily use. The recording mechanism frequently registers 5000 revolutions in the twenty-four-hour period.

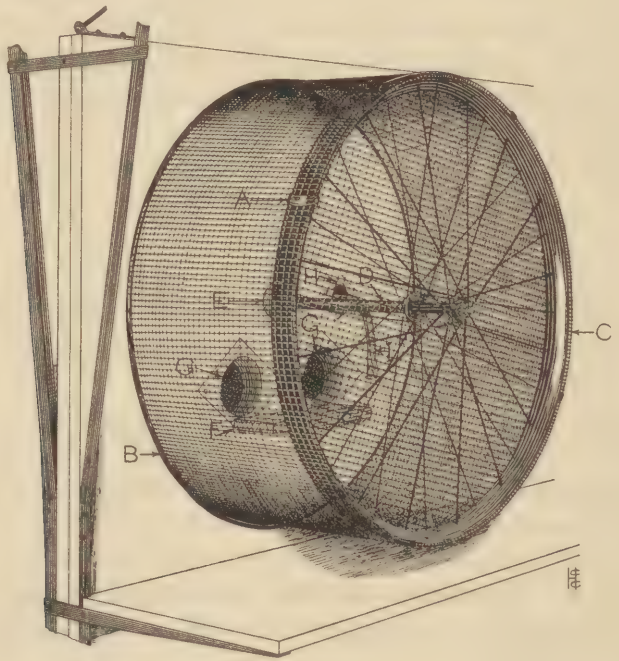


Figure 2

Figure 2 shows one of the revolving cages mounted on a suspended background. The revolving drum is constructed of ¼″-mesh galvanized no. 22 wire cloth. It is 10 inches wide and of the exact diameter to fit snugly over the felloe of a 21-inch bicycle wheel. Here it is held in place by four small brass plates ½″ wide extending across the felloe and secured by wood screws driven into the felloe (*A*). The edges of the wire-cloth drum are reinforced by no. 10 galvanized wire soldered to the outside of the free edge of this drum (*B*), and

to the inside of the edge (*C*) secured to the felloe. The reinforcement of the inner edge affords a shoulder against which the felloe of the wheel bears.

It is essential that the free edge of the drum be rigid and run true with the axis of the wheel.

The end of the axle projecting into the drum is screwed into a ½″ steel rod (*D*), sufficiently long to extend 1½″ beyond the free edge of the revolving drum. The 1½ inches of this steel rod, projecting beyond the limits of the revolving drum, is turned down to a diameter of three-eighths of an inch, forming a shoulder, and threaded for a wing-nut.

This threaded end is inserted into a brass socket (*E*) screwed to the background carrying the revolving cage. It is held firmly in place by a wing-nut on the rear face of the background.

On the background within the revolving drum is a removable wooden shelf, 7″ x 3″ x ⅞″ (*F*), held in place by a bolt extending through the background and shelf. The shelf is removed by unscrewing the wing-nut on the edge of the shelf. The shelf serves as a landing within the revolving drum. The entrances for the rats from the nest box to the revolving drum are through two circular openings 2¾″ in diameter (*G, G*), protected by metal facings. Through another opening 1″ in diameter (*H*), above the end of the shelf, the ⅜″ copper water tube brings drinking water from the bottle on the opposite face of the background, and a drop is always available for the rats at this point (*I*). Excess water drips through the cage to the pan below.

The water bottle used in this cage is described under the heading 'Drinking fountains.'

Each revolving cage is provided with a galvanized steel pan, 18″ x 10″ x ½″ deep, so placed on a shelf beneath the drum as to catch the drips and refuse falling from the drum. Most of this material passes out between the free edge of the drum and the background.

Mounted on the spokes on the outside of the cage near the axle is the recording mechanism with its counterweight. This device, described elsewhere, records the revolutions in both directions.

The nest box (fig. 3), located on the rear face of the background, is 21½″ long, 8½″ high, and 7½″ wide, and is constructed of ½″ thick white pine. It is divided in the middle by a solid wood partition into two compartments. Each compartment communicates with the revolving drum through a 2¾″ circular opening, already shown in figure 2 (*G, G*). The nest box is accessible through the top, which is closed by a lid extending its whole length. In the lid over each compartment a 2″ circular opening covered by a wire screen affords ventilation for the nest box (fig. 3, *A*).

One side of the nest box is closed by the background which carries it. Across this side of the nest box, a ½″ x ⅛″ flat iron bar (*B*) is screwed to the ends and middle of the box, and is utilized to carry the box on two flat hooks, secured to the background. This arrangement makes it possible to remove the nest box easily for cleaning.

Each compartment of the nest box is supplied with a wire-screen floor (*C*), similar to the floor in the dormer cages. These are removable.

Beneath the screen floor a galvanized sheet-steel pan (*D*) serves to catch the drips and particles of dirt falling from the nest box. Above the nest box is located the water bottle (*E*), with its extra long metal tip leading through the background into the revolving drum. The bottle rests upon a small bracket and is held in a vertical position by a wire loop at the top. The label holder (*F*) is fixed on the rear face of the background.

We have used the revolving cages mounted singly on individual stands and in batteries of twelve or less mounted on one large background.

The revolving cage here shown is one of a series of twelve mounted in a double row on a vertical background suspended

from the ceiling. Beneath each row of six cages is a projecting shelf 10″ wide to carry the drip pans under the cages.

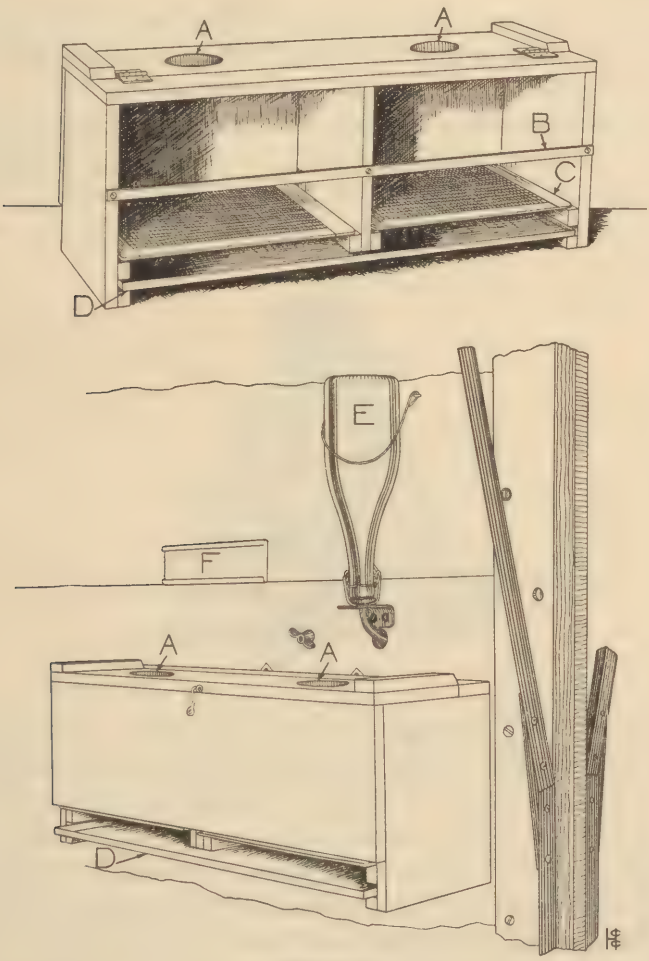


Figure 3

Recording apparatus for revolving cages.—It is desired at times to record the number of revolutions made by the revolving cage. As the rat may operate the cage in either or both directions, it is necessary that the records should indi-

cate the total number of turns made, whether they be in one direction or the other, or part in one and part in the opposite direction.

The counting mechanism made by The Veeder Manufacturing Company of Hartford, Connecticut, and known as Rotary Ratchet Counter No. 6, is used for this purpose.

This apparatus is mounted on a triangular brass base as indicated in figure 4 (*A*). Above the counter is a pendulum (*B*) which swings between two stops (*C*, *C*). One complete oscillation of the pendulum records one revolution on the counter.

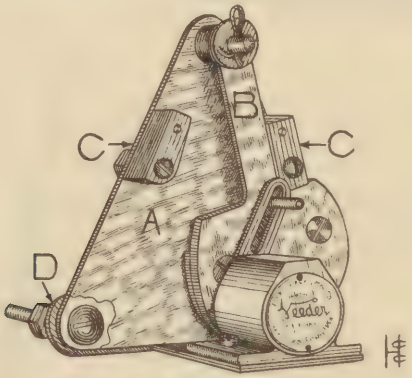


Figure 4

This apparatus is attached to the revolving cage near the axle by means of a clamp (*D*) on the back of the triangular base. A counterweight is used to restore the perfect balance of the revolving drum. Each complete turn of the drum will give one complete oscillation of the pendulum. Two half turns of the drum will also give one complete oscillation of the pendulum and record one on the counting device. Originally we attached a weight directly to the lever of the counter, but it was soon found that the mechanism of the counter is not strong enough to withstand the constant pounding of the weight as the cage revolves.

The turntable.—As a less expensive substitute for the revolving cage, we have used the turntable mounted in a dormer

cage. Its position and construction is shown in figure 5. The turntable consists of a wooden disc ½″ thick and 14″ in diameter.

A modified bicycle wheel hub and axle (*A*) are used to carry the revolving table.

Secured to the lower end of the bicycle wheel hub is a brass disc 4″ in diameter and ⅛″ thick which carries the larger wooden disc.

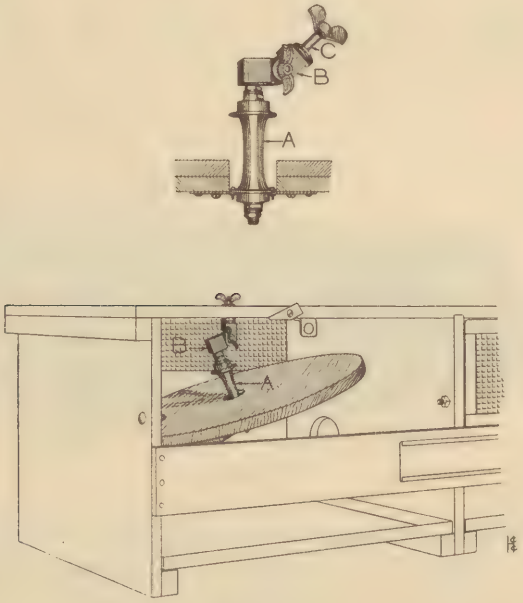


Figure 5

The upper end of the axle is provided with an elbow-joint (*B*), the position of which is controlled by a bolt and wing-nut permitting the turntable to be adjusted to any desired angle.

A stud bolt (*C*) projecting from the upper portion of the elbow-joint passes through the ceiling of the cage and, held in place by a wing-nut, acts as a mounting for the turntable. By removing the wing-nut at the elbow-joint the turntable may be easily removed for cleaning purposes.

While the turntable occupies considerable space within a cage and makes cage cleaning a little more difficult, we are inclined to the belief that it stimulates the rats to a greater variety of exercises than the revolving cage already described.

Armored cages

In case it is desired to house extracted albino rats or wild gray rats, it will be necessary to protect the inner surface of wooden cages, since these strains will gnaw any unprotected wooden surface. For such protection we prefer ¼″-mesh wire cloth.

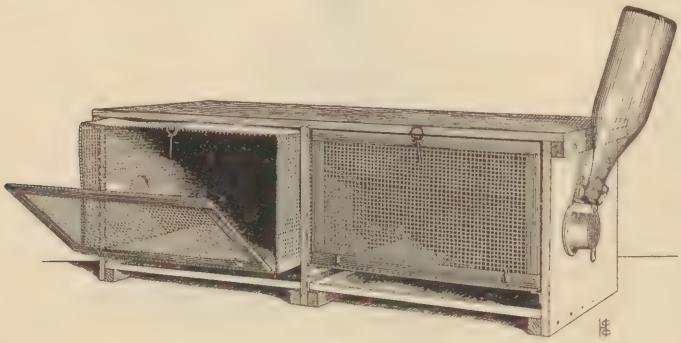


Figure 6

In lining wooden cages with wire cloth, it is desirable to secure the lining to the interior of the cage in such a manner as will permit it to be removed for cleaning purposes. This may be done by fastening a separate piece of wire cloth to each surface of the interior of the cage with screws. Or a removable lining may be made which just fits the interior of the cage, as is shown in figure 6. In this cage the board forming the front wall of the regular dormer cage is omitted. The wire-cloth lining consists here of a complete box with a removable front. This wire box may be removed from the wooden cage without taking the rats from their quarters, provided the openings to the drinking fountain and that to the adjoining compartment are covered. It is convenient to have

sliding galvanized doors to close each of these openings. This is an important feature in handling wild gray rats, which, until they are tamed, are likely to spring for freedom at any opening.

This type of lined cage offers the advantages of security, and for the rats the comforts of the wooden cage.

Other types of cages

While the cages here more fully described are preferred by us for our own work, there are other forms which serve their purpose well, are simpler in construction, and in some instances less expensive.

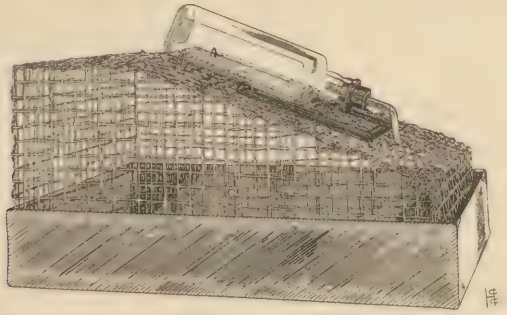


Figure 7

It seems advisable, therefore, to make brief reference to other types of cages, some of which are constructed to meet the special requirements of particular investigations.

One of the simplest forms of metal rat cage is that used by Prof. William E. Castle at the Bussey Institution (fig. 7). This cage consists of a galvanized sheet-metal pan 17″ long, 15″ wide and 3″ deep. In this pan rests the cage proper which is a completely closed box constructed of ½″-mesh no. 18 galvanized wire cloth.

This box is 16″ long, 14″ wide, 8½″ high at one end and 4″ high at the opposite end. For a space of 4 inches wide across the high end, the top is flat, the remaining area of the top slopes from a height of 8½″ to a height of 4″.

In the sloping portion of the top is the only entrance to the cage, 5″ x 5″, closed by a wire-cloth door. Upon the door rests the water bottle of the form shown in figure 13, *A*. The weight of the water bottle is not always sufficient to hold the door closed, an ingenious spring clip is therefore provided to hold it securely in place. These cages will accommodate five or six adult rats. The cleaning process is reduced to a minimum, as the cage containing the rats may be lifted from the pan which catches the litter falling from the cage.

The cages are kept on racks in tiers of five high.

The cages used by Prof. E. V. McCollum, of the Department of Hygiene and Public Health, Johns Hopkins University, are 16¾″ high, 19″ wide, and 24″ deep. A light wooden framework carries the wire-cloth walls of the sides and front of the cage. The back and top are wood. The floor is wood and carries a galvanized sheet-steel pan 1½″ deep. This pan is filled with bedding material and is removable for cleaning. The entire front of the cage is a hinged door. The glass water fountain used is of the type shown in figure 13, *B*.

In Science, N. S., vol. XLVII, No. 1224, June 14, 1918, pages 594-596, Dr. James R. Slonaker describes a very satisfactory metal cage used by him for raising albino rats at Stanford University, California. Slonaker's cage is 24″ long, 12″ high, and 18″ wide. It is divided into two equal compartments and is constructed entirely of metal. Clean water is supplied by means of an inverted bottle similar to that shown in figure 13, *A*.

Investigators who are conducting feeding experiments will be interested in the cages, feeding devices, and methods of handling experimental animals employed by Mendel and Osborne at the Connecticut Agricultural Experiment Station, New Haven, Connecticut. The cages used by these investigators for experimental animals are wire-cloth cylinders 9″ in diameter and 8″ high, closed at one end. An enameled sheet-steel pan, the bottom of which is covered with paper protected by a wire-cloth screen, serves as the floor of the cage.

The feeding and watering devices are placed in the pan with the rats and covered by the cylindrical cage. The labor of cleaning cages and handling animals is reduced to a minimum. The apparatus used and methods followed by Mendel and Osborne in their experiments on nutrition are fully described by Edna L. Ferry in the Journal of Laboratory and Clinical Medicine, vol. V, no. 11, August, 1920, page 735.

We would also like to call attention to the ingenious 'obstetrical cage' of Long and Evans. In their study of the effect of lactation on ovulation and on the corpora lutea, this cage was designed to prevent the suckling of new-born rats and to record automatically the time of their birth.

The cage is provided with a sloping floor of ½″-mesh wire cloth. At the lower side of the sloping floor a slit along the side of the cage permits new-born pups to roll out of the cage and into a box so balanced that the least addition to its weight closes an electric circuit and records on a chronograph the exact time of their escape from the cage. This cage is described in "The Oestrous Cycle in the Rat and its Associated Phenomena," by Joseph A. Long and Herbert M. Evans, Memoirs of the University of California, vol. 6, page 15.

Prof. E. A. Schafer describes in the Quarterly Journal of Experimental Physiology, vol. V, page 204 (1912) a metabolism cage for keeping and feeding albino rats and for collecting the excreta.

The cage consists of a wire-cloth cylinder mounted in a short tin cylinder which terminates in a wide funnel. A wire-cloth disc forms the floor of the cage at the top of the funnel. A short distance down the wide part of the funnel, a brass-wire screen serves to arrest the faeces. The urine is collected in a glass vessel into which the funnel empties.

In The Anatomical Record, volume 11, page 103, Dr. J. A. Long describes the construction of a very ingenious metal cage used in the Department of Anatomy of the University of California for housing rats and mice.

These cages are constructed in groups of twenty and carried on an iron-pipe frame. They may be quickly and easily taken apart for cleaning or sterilizing and readily reassembled. They are most economical of space.

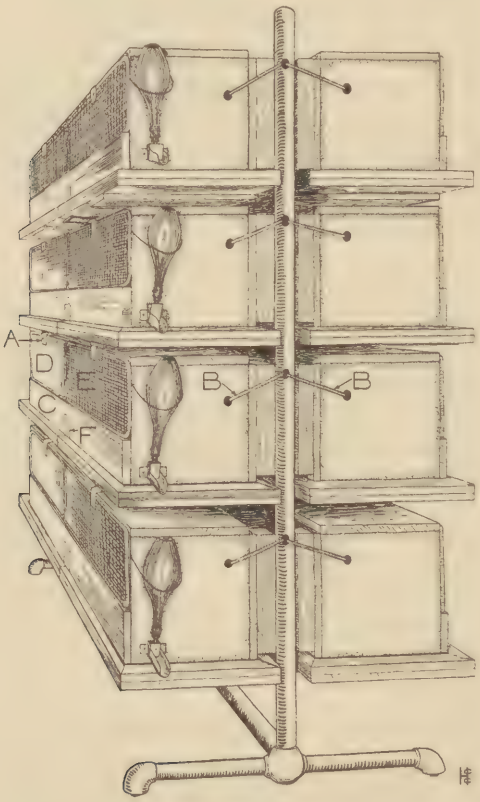


Figure 8

A simple form of cage and cage support is shown in figure 8. This cage is 10″ high, 10″ deep, and 57″ long. The top, back, and floor of this cage are constructed of wood. The lower portion of the front (*C*) is of wood to which are hinged two wire-screen doors (*D*, *E*) opening downward, each of one-half the length of the cage. The doors are held in a closed position by a flat hook hanging over the upper edge of the cage (*A*).

The ends of the cage are of galvanized sheet steel, 10″ x 10″, with a 1″ flange turned on all four edges. To these flanged edges the top, back, and wooden portion of the front of the cage are held by screws.

The floor is in two sections, supported in the middle of the cage by a 1″ x ⅛″ flat iron band extending across the cage (*F*). The outer end of each section of the floor is held in place by a button, engaging the lower flange of the end of the cage. The floor extends sufficiently beyond the limits of the cage to include a ledge ⅞″ high and 1¼″ wide. This ledge prevents the scattering of dirt from the cage.

These cages are mounted on an 1¼″ iron-pipe frame, having two standards drilled to receive the hooks (*B, B*), by means of which the cages are suspended at each end. Each cage may thus be removed without disturbing the others.

The water bottle used for these cages is of the type shown in figure 12, *B*.

Carriers

In the albino rat colony it is necessary to have a number of receptacles at hand where rats may be placed temporarily while making readjustments in the colony or while transferring rats to the laboratory.

For convenience, security, and cleanliness, we have found the ordinary galvanized sheet-steel bucket, modified as shown in figure 9, to serve the purpose admirably as a carrier. A few ½″ holes are drilled in the sides of the bucket about 1½″ from the bottom. The wire-cloth portion of the cover of the bucket is soldered to a no. 10 galvanized wire, bent into hook form at each ear of the bucket. Each hook fits into a small notch cut into an ear of the bucket (*A*). One small clip (*B*) and a bolt through the edge of the bucket holds the screen half of the lid in place. To this the galvanized sheet-steel portion of the lid is hinged. The lid is locked by a clasp (*C*) which engages the rolled edge of the bucket. A standard label-holder is secured to the top.

The entire cover may be removed for cleaning by taking out the bolt at *B*.

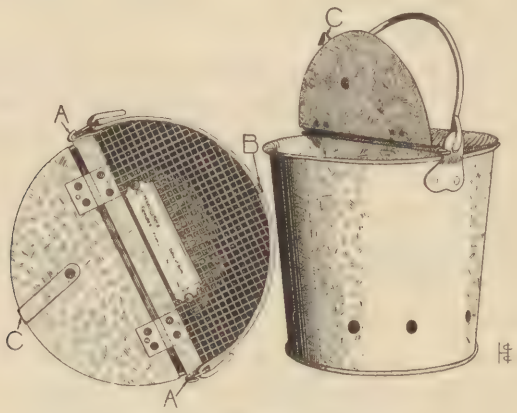


Figure 9

Drinking fountains

The drinking fountain for supplying clean water is shown in section in figure 1.

This device consists of a galvanized cast-iron ring 2″ in diameter, inside, and 2″ long, carrying the inverted water bottle (fig. 10).

One-half inch from one end, this cast ring bears a flange (*A*), ½″ wide. The flange end fits tightly into a circular opening in the end of the cage and is held in place by two screws passing through the flange into the wood of the cage. The cast ring comes just flush with the inner wall of the cage. On the lower segment of the interior of this cast ring at its cage end, a raised edge (*B*) prevents any dripping water from flowing into the cage.

The outer end of this cast ring is closed by a disc of ⅛″-mesh wire-screen cloth (fig. 11). This screen prevents the escape of rats and admits the light. Light is desirable at this point to prevent the rats from filling the space within the cast ring with food, bedding, or other materials contained in the cage.

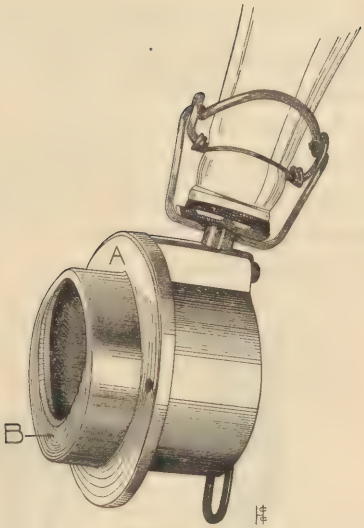


Figure 10

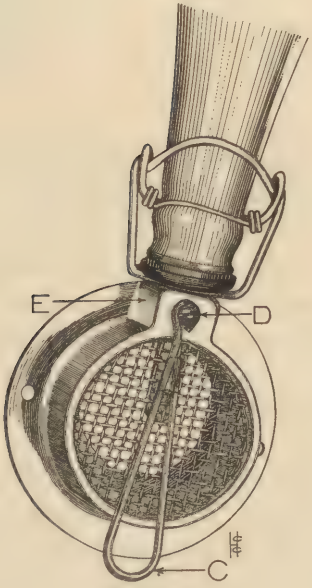


Figure 11

Soldered to the outer surface of this screen disc, a wire loop (*C*) serves to conduct any dripping water outward away from the cage to fall upon the floor. The wire loop and screen disc are held in place by a machine screw (*D*), tapped into a projecting lug at the top of the cast ring.

The lug (*E*) along the upper part of the cast ring is bored to receive the tubular metal tip of the inverted water bottle. This tip extends into the lumen of the cast ring so as to come within easy reach of the rats.

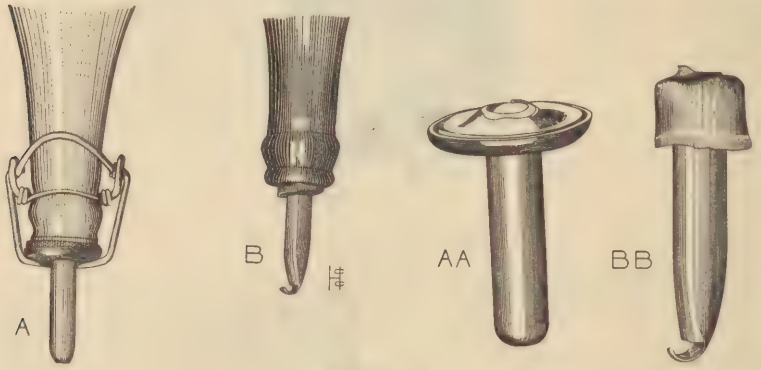


Figure 12

The water bottle which we prefer is a standard grape-juice bottle of one-quart capacity. The bottle is closed by a metal cap, into the middle of which is soldered a ⅜″ brass tube 1¼″ long, the projecting end of which is constricted to a diameter of ⅛″ (fig. 12, *AA*). The cap carrying the tube is made water-tight by a rubber gasket and held firmly in place on the bottled by a modified beer-bottle clamp (*A*).

From the constricted end of the tube the water hangs in a drop within reach of the rats. The opening and refilling of the bottle is easy and rapid. The bottle with its contents is carried by the cast ring. No other support is required.

In attaching the drinking fountain to the cage, it is our practice to permit the bottle to lean about 10° outward and toward the rear of the cage. In this position, drops of water

falling from a bottle above will strike the bottle below at an acute angle on its side, and thus prevent spattering.

When it is desired to supply liquids which should not come in contact with metal, the water-bottle caps and tubes should be made of enameled steel.

For use in revolving cages, where the vibration tends to shake the drop of water from the tip of the bottle, we have found it desirable to use the tip (*BB*) shown in figure 12. This tip is made from 3/8″ (outside diameter) copper tubing. Pieces of this tubing are cut about 2″ long. One-half inch of one end is flattened so that the lumen of the tube is reduced to a slit about 1/32″ wide. One side of the flattened tube is then cut away and the remaining side is curved over the open slit of the tube forming a curved ledge which will support a large drop of water.

The metal tip is held in place in the bottle by a piece of heavy-walled pure gum tubing, or a perforated rubber stopper as shown in *B*. Or it may be soldered into a metal cap and held in place on the bottle by a modified beer-bottle clamp like tip *A*, already described. We have found it desirable in batteries of revolving cages to mount the bottle on the rear face of the background and use a much longer bottle tip extending from the bottle through the background into the revolving drum. The tip or drinking end of the tube in such cases is constructed after pattern *BB*, but held in place on the water bottle as shown in *A*.

Copper tubing tends to destroy micro-organisms in the drinking water; unfortunately, this metal is so soft that the rats sometimes gnaw the tips in a manner to render the tubes unfit for use, and it is necessary in such cases to substitute brass tubing.

Other simple forms of drinking fountains are shown in figure 13.

The bottle with rubber stopper and curved glass tube drawn to a point is intended to lie on the top of a cage with the glass tube extending into the cage within reach of the rats. This is a very satisfactory and easily constructed drinking fountain.

The inverted siphon form of water fountain here shown is made entirely of glass and is intended to hang on the side of a cage in a vertical position. Water is always available at the open end. This water cup is excellent because of its one-piece, all-glass construction. It is usually manufactured of a size to hold about 125 cc. of water. It is, however, open to the objection that the water may be more easily contaminated.

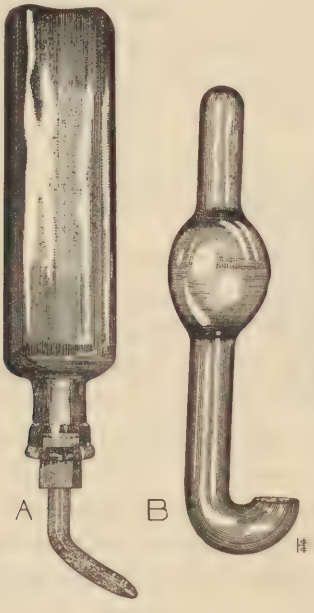


Figure 13

Feeding devices

In quantitative feeding it is essential to have a food container or feeding apparatus which will allow the rat to secure all the food it will eat without permitting the remaining food to be scattered about and mixed with the litter of the cage.

Several forms of apparatus have been devised for this purpose.

The feeding device shown in figure 14 is a form used by Prof. E. V. McCollum, of the School of Hygiene and Public

Health, Johns Hopkins University. It consists of an outer tin pan (*C*)—an ordinary cake pan—8″ in diameter and 1¼″ deep. In the middle of this pan a plain tin cup (*D*), 3½″ in diameter and 2½″ deep is held in place by a wire loop attached at two points to the edge of the pan. This wire loop permits the cup to be removed for cleaning.

The cup is covered by a circular disc of tin, 6″ in diameter, with a central hole 1⅛″ in diameter (*E*). This tin disc is

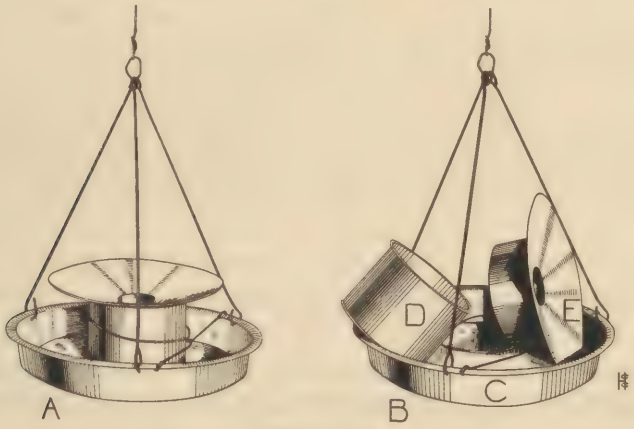


Figure 14

slightly convex, dipping towards the central hole, forming a funnel-like top to the cup. A circular flange 1¼″ wide is soldered to the underside of the circular disc. This flange fits accurately into the cup and holds the disc in place.

The whole apparatus is suspended by three wires attached to the edge of the pan and brought together in a ring 10 inches above the pan. From this ring a single wire suspends the apparatus from the ceiling of the cage.

Food is placed in the cup. The rats may stand on the disc and take food from the cup through the central opening in the disc. Food not eaten tends to fall back into the cup or if scattered over the edge of the disc is caught by the pan below.

Another 'self-feeder for rats' is described by Ralph Hoagland, Bureau of Agriculture, Washington, D. C. This feeder consists of a vertical feed hopper with a horizontal wire-screen vestibule of sufficient size to permit only one rat to approach the feed hopper. Beneath the apparatus is a pan for catching waste feed. Hoagland's device is accurately and fully described and figured in the Journal of Laboratory and Clinical Medicine, vol. 7, no. 11, August, 1922.

Feeding devices of this type are serviceable when a more or less dry and granular or powdered food is supplied.

In supplying rats with a varied diet, such as we have here described, feeding devices are of little use. Small dishes of tin, earthen-ware, or glass have served our purpose. Much of the food is placed directly on the cage floor—a method of necessity, rather than choice—since a considerable proportion of the food thus supplied is wasted.

As yet we have been unable to devise a feeding apparatus which may be used to feed all the various forms of food supplied to our colony. This is due chiefly to the instinct of the rat to hoard food. If it is possible, the rat will take food from a receptacle of any kind and store it in the dark corner of its cage or cover it with litter, or if sufficiently plastic, may use it to close a crack along the floor of the cage to stop an uncomfortable draft. Much of the food thus stored away by the rat becomes unfit for its consumption and is wasted.

SPECIAL EXPERIMENTAL STRAIN OF ALBINO RATS

In the discussion of food, behavior, and breeding, it should be remembered that we are using observations on a special strain of albino rats which were bred and reared under unusually favorable conditions.

The single pair, from which the strain was bred, was selected from the standard stock of The Wistar Institute Colony.

This strain was developed and used in the course of a series of experiments intended originally to determine the best practical methods of producing satisfactory animals for research purposes.

A large amount of data bearing on growth, fertility, hygiene and behavior was accumulated and remains yet to be published.

Hartwell, Mottram and Mottram ('23) have described a technique for the production of a good stock of large rats for feeding experiments. Certain features of their methods correspond to ours.

In suggesting foods and methods of preparing foods, we have in mind the problem of producing normal, vigorous albino rats, and not the development of any special strain or the rearing of albino rats for any special investigation. In the breeding and rearing of albino rats for research purposes, nutrition is one of the important processes which determine the degree of success of the undertaking.

Suitable food is not alone sufficient to secure well-developed animals. Conditions must prevail which will induce the rat to partake of its food, to digest and assimilate it. With the foods here suggested and the methods of care described, albino rats of unusually fine physique and of large size were produced. Furthermore, the fertility of our experimental series was increased more than 40 per cent above the recognized standard, and sterility was reduced to a marked degree. The effect on body weight of the food and methods here described is shown in chart no. 1, where the growth curves of body weight of our experimental stock are plotted with those of standard stock.

That animals must be nourished in order to grow is common-place information. That food may quickly produce morphological changes in the internal organs of the rat and seriously interfere with normal functioning is a fact not so generally understood.

A distinctly deficient diet is likely to show its effects in one of several forms of malnutrition. In our experience, a failure to grow, eczema, xerophthalmia, and sterility have been the more common results of deficient diet.

A diet may produce an animal with most of the outward appearances of a normal albino rat, yet anatomically, physiologically, or chemically it may not comply with the exacting specifications established for a research animal.

The anatomy and the chemistry of the albino rat are quickly and profoundly modified by its food supply. Parasites, both internal and external, seriously influence nutrition. The inherited characters of the animal, its acquired immunities or the lack of them, add to our problem a series of factors which are in the beginning unknown and most of which must for the present remain unknown.

The serious deviations from normal growth due to improper food and to parasites led us to attempt to select food containing all the essential elements for the promotion of growth in the albino rat, and to prepare this food in a manner to avoid the ingestion of living parasites and furnish a ration which the animal would readily accept and enjoy.

While reasoning by analogy may be suggestive, it is unsafe to assume that the diet which produces a good, healthy, normal animal of one species will prove equally efficient in another species.

In addition to food of the proper kind, considerate attention is necessary in producing good vigorous animals and in maintaining a high degree of fertility.

In selecting and preparing food for the albino rat it has been our experience that a so-called balanced ration does not prove effective for long and continuous use. This may or may not indicate the lack of some essential food element. The rat apparently enjoys changes in his diet.

It was assumed in the beginning of our experiments (results to be published elsewhere) on the production of albino rats for research purposes that, beginning with a clean, healthy stock and a uniform diet with uniform environmental conditions, we could produce a standard or uniform animal within accepted limits of variation.

The results were not what we had expected. Even with a diet as well balanced and uniform as may be devised, with environmental conditions as uniform as may be secured, there are climatic conditions presenting varying conditions of light, humidity, temperature, atmospheric pressure, together with

noises, odors, changes in the manner of caretaking, in the personnel of caretakers, and many other conditions of a subtle nature which influence the growing mammal. All these, together with the variable and undeterminable state or condition of the albino rat itself in its reaction to food and to external influences, result in a constantly changing organism.

It is highly important in maintaining animals for experimental purposes to bear in mind these influencing factors that they may be made as uniform and as constant as possible.

Experiments should be controlled by animals of the same age and weight and litter, if possible, which have been reared at the same time and under the same conditions as the test animals. Sex should be considered, since the two sexes do not react in like manner. Likewise, two strains of the same species may differ in their behavior. Caretakers should not be changed in the midst of experiments, otherwise irregular or inexplicable results may be obtained. This is especially marked with timid animals or the less domesticated strains.

The diet which we have used successfully is a highly varied mixture of available cereal grains, roots, tubers, legumes, green leaves, red meats, fish, nuts, seeds, fruits, milk, eggs, fats, sugar, starches and some prepared foods like pilot bread, macaroni, soda crackers, dog biscuit, and shredded coconut, and the long list of breakfast foods.

We have assumed that the rat will exercise his instinctive ability to choose from an ample supply of varied foods, those foods most essential for his nutrition. There is experimental evidence to support this assumption.

Furthermore, it is quite possible that our knowledge of dietary essentials may be incomplete and that by furnishing a constantly changing variety of foods, there will be less danger of omitting some essential food element hitherto not recognized.

Sources of food supply

As the albino rat thrives on many foods consumed by man, the sources of supply are practically identical with the sources of human food supply.

Like the supply for man, seasonal variations are necessarily introduced. At first thought one might easily believe that a colony would thrive best and produce animals more nearly uniform or standardized in a semitropical country where the food supply may be more uniform throughout the entire year. This, however, raises another interesting problem of the effects of seasonal changes upon an organism.

Clean table scraps, when obtained in a fresh condition, form an excellent ration. In this material a large variety of foods usually occur. Bits of meat, bone, fat, cooked vegetables, celery tops, grape-fruit rinds, bread, cake, lettuce, fruits, eggs and milk as constituents in pastry give to this waste product a food value for the rat. It is advisable to supplement table-scrap diet with those foods that appear to be absent or to occur rarely. Such a food supplemented with milk and green vegetables like kale, chard, lettuce, or cabbage, forms a very satisfactory ration in maintaining a small colony.

To furnish table scraps for a large colony is not always easy, nor is it altogether satisfactory when such material is collected in large quantities. When collected in considerable quantities it should be sterilized at once by cooking. Even when collected from private family tables it is safer to sterilize the food scraps before feeding them to the rats, thus obviating infections from this source. Unfortunately, the second cooking probably reduces to some degree its value as a food.

It has been our experience that table scraps are too unreliable both as to quantity and quality to be depended upon for a colony of several thousand rats. We, therefore, depend upon the wholesale grocer, the wholesale dealer in grains and feeds, the flour mills, the produce dealers, and the meat and fish markets for our supply of rat foods.

Milk and eggs are purchased in powdered form from the wholesale firms. Fresh whole milk, when it can be obtained and used in fresh condition, is to be preferred to powdered

milk—it has lost none of its food factors. The same is probably true of eggs, although we have used powdered eggs (imported Chinese) with success for some years past. If they have lost any of the food factors originally contained, these probably have been supplied from other sources.

The food storeroom of the colony should be provided with tight, preferably metal, containers for storage of some of the foods. It is essential that stored foods are not accessible to wild mice, rats, or other animals. The danger arises chiefly from the parasites these animals may carry.

The well-supplied storeroom should contain whole wheat, as well as its various products, such as cracked wheat, whole wheat flour, wheat germ and middlings; oats and their prepared form, such as rolled oats; corn-meal or cracked corn, hominy grits, barley, linseed-oil meal, rice, dried lima and navy beans, dried split and whole peas, canned mixed soup vegetables, canned salmon, pilot bread or hard tack, macaroni, meat scrap or powdered beef, egg powder, whole milk powder, powdered clean fresh bone and salt, preferably crude sea-salt. Potatoes, turnips, carrots, and beets may be stored if a suitable cool place is provided. It is our practice to purchase potatoes in the autumn for the winter's supply. Other tubers together with fruits and green leafy foods mentioned are purchased as needed.

Preparation of foods

The following methods have been successfully employed in preparing combinations of foods that have produced healthy albino rats of large size.

Our object in combining various foods, as here described, is not only to furnish a variety of food elements, but also to produce combinations which are attractive to the rats. Our experience indicates that albino rats do not enjoy and thrive as well on a monotonous diet.

Wheat and peas with milk. Take 1 part, by bulk, of dried whole peas or split peas, or preferably ½ part of split peas

and ½ part of whole peas, soak for from five to ten hours in cold water, then add 3 parts of whole wheat and sufficient salt to season. Cover with water and cook in the steam kettle until soft, adding water from time to time as required. When cooked the mixture should be soft and comparatively dry, all excess water having been absorbed or evaporated. Remove to large trays, and when cool pour whole milk over the mixture, using at least 1 quart of milk to every 8 quarts of food.

Wheat and beans with milk. Take 1 part of either dried navy, lima, or split beans to 3 parts whole wheat by bulk. Soak the beans in cold water for from five to ten hours, then add the wheat and sufficient salt to season. Cover with cold water, stir to mix, and cook in a covered steam kettle until both are soft, adding water from time to time as necessary. When done this mixture should be comparatively dry, all excess water having been absorbed or evaporated. About four hours' time is required to complete the cooking. Spread the mixture on large trays to cool. When sufficiently cooled, pour whole milk over the mixture, using at least 1 quart of milk to every 8 quarts of food.

The addition of a large split soup bone occasionally to the wheat and beans or to the wheat and peas gives a rich flavor to these combinations. The bone is allowed to cook with the mixture from the time cooking begins, and when finished the bone may be given to the rats to gnaw.

Barley, salmon and eggs. To 8 quarts of barley add sufficient salt to season, cover with cold water, cook slowly in covered steam kettle until the barley is thoroughly done, adding more water as needed from time to time. When thoroughly cooked, the barley should be soft, comparatively dry, and have no excess water in the kettle. To this add 2 one-pound cans of salmon and ten or twelve eggs well beaten, or ¼ pound of egg-powder previously mixed with a pint of cold water. Stir this mixture of barley, salmon, and eggs all together while hot until evenly mixed and then spread on trays to cool.

Meat, rice, and macaroni. To 8 quarts of rice add 1 pound of macaroni broken into small pieces. Cover with cold water and allow to soak for about two hours. Take 2 pounds of brisket containing some fat, and salt to season; add sufficient water to cover it and cook slowly in covered steam kettle until the meat falls apart and is easily shredded. Add this shredded meat, also the water in which it was cooked, to the rice and macaroni previously soaked for two hours, and boil all together in the steam kettle until thoroughly cooked, adding more water when necessary. The rice should be soft and flaky and the entire mixture comparatively dry, all excess water having been absorbed or evaporated. Spread on large trays to cool. White potatoes may be substituted for the rice, or part rice and part potatoes used, or potatoes and turnips in about equal parts may be used, either alone or with rice.

Hominy grits, vegetables, and eggs. To 8 quarts of hominy grits add sufficient salt to season and cover with cold water. Of fresh vegetables take corn cut from the cob, cabbage, kale, celery, cauliflower leaves, tomatoes, carrots, parsnips, turnips, potatoes, squash, and egg plant, or any combination of these that are obtainable at one time, and cut into moderately fine pieces. Add to the hominy grits about 2 quarts of these cut vegetables and cook in the covered steam kettle until the hominy grits are soft and all excess water is absorbed or evaporated. To this add ten or twelve eggs, well beaten, or ¼ pound of egg powder, previously mixed with 1 pint of cold water. Stir the eggs into the cooking mixture, remove to large trays to cool. If fresh vegetables are not available, substitute canned mixed soup vegetables, adding 2 one-pound cans when the egg powder is added, after the hominy grits are thoroughly cooked.

To the above mixture may be added 1 quart of beans, either dried black beans or dried lima beans. These should be cooked first for several hours, until soft, and then run through a meat chopper. When using the beans the eggs may be omitted. Stir well to mix and spread on large trays to cool.

When this mixture has been poured on the trays, sprinkle over it a thin layer of shredded coconut which should be pressed down into the soft mixture, in order that the coconut may be uniformly distributed to the rats.

Fish and corn-meal. A cheap fresh fish such as haddock is very satisfactory. Five pounds of cleaned fish with the heads on are placed in the covered steam kettle, sufficient salt to season and enough water to cover is added. Cook until the fish falls to pieces, then gradually pour in 20 pounds of coarse yellow corn-meal, adding more water if necessary, cook a few minutes to thicken the meal or until it is stiff enough to be cut down in slices to feed. Spread on trays to cool.

Rolled oats, wheat, beans, rice and milk. Allow 2 or 3 quarts of dried lima beans or dried black beans to soak overnight, then cook until soft. Add 3 quarts of rice, 3 quarts of whole wheat, and 3 quarts of rolled oats, with sufficient salt to season, and cover the entire mass with water and cook until all the ingredients are well done, adding water from time to time if necessary. When thoroughly cooked, allow any excess moisture to evaporate. To the well-cooked mass add as much whole milk as it will absorb. Pour on large trays to cool.

Soy beans, rice, and meat. Soak 8 quarts of soy beans in water overnight, place these in the cooking pot with 3 pounds of beef brisket and 3 pounds of smoked ham, add sufficient salt to season. Cook all together until the beans are soft—from two to three hours. Run this mixture through the meat chopper, then add 6 quarts whole rice and 1 pound of sugar and continue cooking until the rice is well done. Pour out on large trays to cool.

Fish, corn-meal, peas, wheat, and ham. Cook the string end of a ham until thoroughly done, several hours, depending upon its size. Dried peas, either whole or split, may be cooked with the ham. When cooked, cut the ham up fine or run it through the meat chopper.

The minced ham is then returned to the pot containing the peas and the liquor in which they were cooked. Add 1 part

of whole wheat and 5 pounds of fresh fish, such as haddock, cooking all together until the fish falls apart and the wheat is soft—usually about three-fourths of an hour to an hour.

To this add gradually 20 pounds of coarse yellow corn-meal. Cook until it thickens, adding more water if necessary. Pour on large trays to cool.

Rice pudding. Cook 8 quarts of rice in water until well done, then add 1 dozen fresh eggs well beaten, 2 pounds of brown or granulated sugar, 2 pounds of raisins and 4 quarts of whole milk, or as much as the rice will absorb and be of the right consistency to feed. Stir this well to mix all the ingredients evenly without further cooking. Pour on large trays to cool.

To this mixture may be added ¼ pound of cooking chocolate previously melted in a small amount of hot water.

Liver. Liver may be cooked with other foods in place of meat, or it may be prepared separately as a dry granular mass to be mixed with any of the cereal grains.

To prepare liver in a dry form: Cut the liver into slices about ¼ to ½ inch in thickness, place in boiling water and boil for about five minutes or until all the tissues are firmly coagulated. When cold pass the boiled liver through a meat grinder, spread on paper, and dry in a steam-heated or other suitable drying apparatus.

The resulting mass will crumble to powder and may then be mixed with a dry cereal, using 5 per cent or less of the liver powder in such a food mixture. Rats are especially fond of liver in this form.

The feeding of liver must be done with caution, as it may produce excessive intestinal secretions and a condition of diarrhoea.

Oats, middlings, and wheat germ, with milk. Take 4 parts by bulk of oats (with hulls), 4 parts of wheat germ, 2 parts of middlings, 1 part of sugar, brown or granulated white. Place the oats in the covered steam kettle, add sufficient salt to season, and cover with water. Boil until the oats are soft,

then add the middlings, wheat germ, and sugar and cook until of a stiff mush-like consistency. Whole milk may be added to this, as much as it will absorb, or it may be fed without the milk.

Rolled oats and corn-meal, with clabbered milk. Rolled oats 1 part by bulk, corn-meal 2 to 3 parts. To 1 part of rolled oats add sufficient salt to season and enough water to cook to a jelly-like consistency. Allow this to cook in covered steam kettle with very little steam overnight, if possible; if not, for six to eight hours; then add 2 to 3 parts of corn-meal, and cook until it thickens, stirring to mix and adding more water if necessary. Place on large trays to cool. Take whole milk, allow it to sour, and pour into a cheese-cloth bag to drain. Save the whey by suspending the bag over a bucket or any large pan, and use this liquid in cooking other foods. When drained, remove the curds from the bag and pour over the trays of oats and corn-meal, mixing in either with a large spoon or with the hands. Any quantity of milk treated this way may be used.

Post Toasties, hard-boiled eggs, and shredded coconut. Boil eggs hard, then with a fork break up both the white and yolk and mix all together. Season with salt. Sprinkle this over Post Toasties and feed in pans or dishes. Shredded coconut may be added to this occasionally.

Any fresh vegetable or any combination of vegetables added to one of the mixtures mentioned and cooked with it will enhance its food value and also appeal to the rats' appetite.

All foods prepared according to the foregoing formulas should be used at once while fresh.

In addition to the combinations of cooked foods as above described, fresh fruits and vegetables, such as oranges, grape fruit, apples, pears, canteloupes, watermelons, plums, peaches, lettuce, kale, cabbage leaves, celery leaves or celery stalks, Swiss chard, tomatoes, and carrots, should be fed raw with any of the cooked combinations mentioned. All uncooked fresh fruits and vegetables should be thoroughly washed in cold water before feeding.

Milk and milk powders. Fresh whole milk is best to use when available. In the conduct of a colony, however, it is not always convenient to secure fresh milk when needed, under such circumstances the powdered milk may be used. It can be obtained from wholesale dealers in convenient-sized packages and will keep for a long time if stored in a dry and moderately cool place. In this form milk is available at any time, and while not as good as fresh whole milk, it has given very satisfactory results.

Whole milk powder is reconstituted as follows: For every gallon of cold water use 1 pound of the whole milk powder. Put the powder on the top of the water and mix thoroughly using a rotary egg-beater or whip. It is recommended by the manufacturers to reconstitute the milk several hours before the time it is to be used.

Eggs and egg powder. What is true of milk powder may be said of egg powder. Fresh eggs are to be preferred. One pound of egg powder is equivalent to about forty eggs. In preparing egg powder use 1 pound of the egg powder to about 3 pints of cold water. Place the powder on top of the water and mix thoroughly with a rotary egg-beater or whip. This solution of egg powder may be cooked in mixtures of different foods as described elsewhere, or it may be cooked slowly on a range, stirring constantly until it has coagulated, having the appearance of scrambled eggs. After seasoning with salt it may be fed in this form either alone or with other foods.

Raw meat may be fed occasionally with advantage. It should be fresh and prepared by cutting into small pieces or running through a meat grinder.

Large bones, especially the ends of long bones, afford a convenient supply of mineral salts and give the rat a certain type of exercise desirable for the proper secretion of digestive fluids. Crab shells, lobster shells, and oyster shells may also be utilized to furnish mineral salts. These substances probably excite the appetite to some extent and thus aid in the digestive processes.

In addition to the food preparations mentioned we have found the following ration, suggested by Prof. E. V. McCollum, to which we have added salt and bone powder, to be of use.

Thirty pounds of whole cracked wheat, 30 pounds of rolled oats, 30 pounds of corn-meal, 10 pounds of flaxseed meal, ½ pound of salt, 1½ pounds of powdered bone.

These ingredients are thoroughly mixed in the dry state, then poured into sufficient boiling water to make a dough and allowed to cook for a half hour in the steam kettle. The dough-like mass is allowed to cool and is then run through a meat grinder having a perforated cutting disc with holes about ¼″ in diameter. The mass comes from the machine in long macaroni-like threads, is placed on wire-cloth screens, ¼″ mesh, and dried in a steam-heated drying oven. The ration thus prepared will keep in a dry state almost indefinitely, and on this account is a desirable food to have on hand in case other foods are not available. We have designated this diet as 'McCollum mixture.'

This ration is fed in the dry state or in dishes with a little whole milk. If fed for any length of time it should always be supplemented by milk.

As an occasional food it may be freshly prepared and used in the moist condition without grinding and drying. It should be fed with milk. The milk may be poured over the mass which will absorb a large portion of it or the mass may be fed in dishes with milk.

It is one problem to supply a food, and quite another to induce an animal to eat sufficiently of a given food to thrive. For this reason the various combinations have resulted. It is desirable to produce a mixture which the rat enjoys.

While the foods mentioned have been used for our normal animals, it is occasionally desirable to prepare special diets when for one reason or another an important animal becomes ill and it is desirable to nurse it back to health. In such cases warmed milk, sweetened cocoa made with milk, and cod-liver oil have been used with success.

Water

It is desirable to have constantly available for the rats a supply of clean water. We have considered it advisable to boil all the drinking water when the supply was not considered safe for human consumption. As the rats invariably foul the drinking water if exposed in open vessels in cages, it is better to use drinking fountains which will protect the water supply from contamination.

Feeding

Good results may be obtained by feeding the rats of a colony once each day, giving a double portion on Saturday and omitting the feeding on Sunday.

As the rat is a nocturnal animal, taking most of its food during the night, the single meal each day should be given late in the afternoon, after the cage cleaning and other work of the colony has been done. At this time of the day the rats become more active. The rattle of the food trays and the noise of the attendants in distributing food tend to increase their normal activities and excitement.

They exhibit the greatest interest in the feeding process. In a properly conducted colony a failure to do this indicates illness or fear. In either condition growth cannot be expected.

They eat heartily, romp and play about their cages. This process of alternate feeding and exercising continues until late into the night.

In the morning the rats are either asleep or listless. They spend much of the day in a more or less dormant condition.

Better growth and increased fertility may be obtained by feeding twice each day. Under such a regime, a greater variety of food may be supplied, resulting in greater consumption.

In following this method, a simple small ration should be given in the morning. This ration may consist of dry pilot bread, or pilot bread soaked in whole milk, with or without sugar, or raw carrots or lettuce or other green leaves, and

dry McCollum mixture, or chopped hard-boiled eggs and one of the cold prepared cereals.

Shredded coconut may be given occasionally with the morning meal.

The heavier evening meal may be selected from the list of foods described under 'Preparation of foods.' Both the morning and evening ration should be varied daily.

A supply of clean water should always be available.

The amount of food required by each rat will vary according to age. The young growing rat (60 to 100 days) should be supplied with a greater quantity than the adult.

In order to supply all the essential food elements for rapid growth and proper development, the young rat should be fed an abundance of highly varied diet. Roughly 80 grams daily of any of the suggested cooked food mixtures will be ample for a growing rat. And this allows for a considerable waste which always occurs in supplying food in this form.

The actual amount consumed by one rat may not exceed 20 grams in one day.

As a rule, the adult male eats a little more than the adult female when she is not breeding.

It is well, after serving each cage with its allotment of food, to look over the cages carefully to see that the food supply is ample. Occasionally a rat or a group of rats will be found which eat much more than others, and such big feeders should be well supplied, especially during the period of rapid growth.

Feeding breeding rats

The success of the colony depends largely upon the attention bestowed upon the breeding rats, especially the breeding females. In feeding the breeding rats, it is assumed that one male and one female will be caged together.

It is well worth while to pay special attention to the rats which have been selected as breeders. Extra food and a greater variety to tempt the appetite will fit them for the breeding process.

In addition to the regular cooked food supply, it is well to furnish prospective breeders with additional milk served with cracked whole wheat or other cereal, previously cooked, or whole wheat bread, and a green vegetable or fruit daily, such as lettuce, raw carrots, raw cabbage, or oranges, apples, or pears.

In cold weather the milk and wheat or other cereal should be given warm. In fact, all other foods are more acceptable to them if warm.

The pregnant female appears to be always hungry and devours everything offered her. Advantage should be taken of this condition to supply her with all she can consume. She eats much more during pregnancy than the male. What she does not eat immediately she is careful to stow away in her cage, and usually it is all consumed before the next feeding time. This increase in appetite is more marked as the pregnancy advances.

About twelve to twenty-four hours before the birth of the litter, some females eat very little. Whether they are uncomfortable or in pain is not known, but they do not behave as usual.

Occasionally, following the birth of a litter, a female appears sick, her fur will present a chalky-white appearance and stand out stiff and wiry from her body, her eyes will appear dull and dark, her face will seem pinched, and her back arched more than normally. Cases of this kind frequently recover quickly if given warm whole milk or sweetened cocoa made with whole milk, with soft food such as bread or hominy or cooked barley, three or four times during the day. A few drops of cod-liver oil mixed with the food is useful for such cases.

Feeding the lactating rat

The care of the nursing mother is as important as the care bestowed upon her during pregnancy. The tax upon her increases continuously as the pups increase in size.

When the litter is large, twelve or more, the female should be fed more frequently (three or four times a day) or a greater quantity at each feeding. The supply of whole milk, cooked whole wheat, meat, green raw vegetables and fruits, such as lettuce, chard, cabbage, kale, carrots, oranges, and apples, should always be available to the nursing mother. During this period the mother is constantly feeding. She seems to enjoy sweet foods especially.

The larger the number of pups in a litter, the greater appears to be the stimulus for milk production by the mother, but she must be amply supplied with a varied diet to meet the demands upon her.

Feeding the newly weaned rats

Nursing young are usually weaned when thirty days of age. At this time they may be expected to thrive without the mother if properly fed. If the lactating female is again pregnant, it is advisable to wean her pups when they are twenty to twenty-one days of age, in order to give the mother rat an opportunity to rest and accumulate a little excess nourishment before the new litter is born.

As a rule, when pups are not removed from the mother before the succeeding litter is born, the older litter will get most of the new milk supply. Occasionally, the mother rat will make a nest for the new-born litter and will not permit the older litter to nurse.

During the latter part of the nursing period the pups will begin eating some of the milk and soft food supplied for the mother.

The weaned rats should be given ample space for exercise. A roomy cage permits them to run and play. A turntable mounted in the cage furnishes extra stimulus for exercise and will aid in vigorous development.

When caged alone their food should consist of whole milk with a cooked cereal, such as whole or cracked wheat, rolled oats, or hominy. This should be fed in small dishes so that

an extra quantity of milk may be supplied. With the milk and cereal should be fed a small amount of lettuce.

During the first thirty or forty days after weaning, the pups should be fed twice each day.

Clean water should always be available.

Gradually, as the pups approach sixty days of age, the food supply may be modified to include, in small amounts, the heavier diets described under 'Preparation of foods,' and the feeding of milk and soft foods may be reduced in frequency and amount until the animals are fed the regular colony diet.

Feeding the developing rat

From 60 to 150 days of age the developing rat requires the most food of any period during its life-span. During this period, the foods suggested should be utilized, taking care that the same food is not used on successive days. Ample amount, highly varied, appears to be the motto for feeding at this period.

It is well to have constantly in each cage a few large crystals of common sea-salt and some pieces of oyster, lobster, or crab shells, or pieces of a large bone. This assists in keeping the teeth in good order.

BEHAVIOR

Under this heading, we have placed such information, relating to the activities and reactions of the developing rats in the colony, as concerns their vigor, fertility, and general well-being. As in the case of other domesticated animals, it is not sufficient merely to feed and water albino rats and keep their cages clean.

Individual attention, shown by handling and petting, is essential for the best growth of albino rats and for securing uniform reactions when used as research animals. They should have ample opportunity to know their caretakers. Contentment soon replaces fear when they are placed in cages where they may receive some individual attention and where they may observe the various activities of the colony house, become accustomed to the noises of the place, and hear the voices of those engaged in the work of the colony. Under such conditions, they eat and assimilate food in a more satisfactory manner. The absence of fear permits them to feed with pleasure and to digest food with none of the inhibiting influences of nervous tension which tend to restrain digestive secretions. Under such conditions, fertility is markedly increased.

Albino rats placed in dark and unfrequented corners of the building become timid and are easily frightened. They do not eat well. They shun contact with caretakers and seek seclusion in the dark corners of their cages when any one approaches. They do not thrive. They may breed occasionally, but fertility is distinctly below normal.

The difference between the timid albino rat and the gentle albino rat is shown in a most impressive manner by the fact that more than 75 per cent of gentled albino rats will survive the removal of the parathyroid gland, while less than 15 per

cent of timid or ungentled albino rats will survive this operation. The mechanism of this reaction remains yet to be explained.

The effect of gentling is also shown in the reaction of the intestinal muscle segment to sodium carbonate stimulation. Such a segment from a gentled rat will always respond by a definite contraction, while a segment from a timid or easily excitable rat will give a reversed or irregular reaction to such stimulation.

These observations indicate how essential it is, especially for certain experimental work, to have gentled animals. There is also a time-saving element in producing parathyroid-less animals at the rate of 75 per 100 instead of 15 in every 100 operations.

Taming the colony or maintaining it in a condition of fearless contentment is not only economically desirable, but scientifically essential. It is also a time-consuming task.

In handling rats, especially when they are unaccustomed to such treatment, one should approach the animals very slowly and quietly, making no quick or unexpected movement.

Do not pick up a rat by its tail if this can be avoided.

Grasp the rat gently about the body with its head towards the palm of your hand, closing the fingers under the abdomen, with the little finger under the rat's jaw. Any attempt of the rat to bite you may then be thwarted by closing your fingers firmly on its throat.

Once held in your grasp for a moment or two, the rat will relax and very soon become quite contented in your hands. After such daily treatment for a brief period the rat will display no fear and will be quite satisfied to be handled. Albino rats are likely to exhibit greater fear when approached in the darker retreats of their cages. They seek shelter in such retreats and resent any attempt to remove them. The same animals may be approached with less risk of offense when they are out in open space.

Even in a well-ordered colony it must be remembered that disturbances, such as unusual noises, changes in the routine of the colony work, visitors in the colony, and changing the caretakers, will so influence the rats as to interfere with normal processes of digestion and metabolism, and there will result a condition resembling brief starvation. This condition, possibly by its inhibiting influence on the metabolic processes, may result in delayed ovulation and seriously interfere with breeding. One of the serious disturbing events which may happen in a colony is to change the caretakers. Like other domesticated animals, albino rats are sensitive to strange attendants, and until they become accustomed to new attendants, variations in their performance may be expected. The most easily observed deviation from normal behavior under such conditions is retarded growth.

In conducting any series of experiments with albino rats which are being frequently weighed, results are likely to be misleading if during the period of observation there is a change of caretakers or anything occurs to disturb the regular routine of the colony.

Chart no. 2 shows the growth curve of twenty-five cage-bred wild gray rats.

During the interval shown on the chart between *C* and *F* there was an unusual disturbance in the colony caused by the shifting of cages and the delousing of rats. This particular series failed to gain in weight as before and after the disturbance.

Chart no. 3 shows the growth curve of one male and the combined growth curves of two females. The interval shown between *L* and *M* on this chart shows the effects of the disturbance in the colony above referred to, while at *B* the effects of the loss for a time of the regular caretaker are shown.

Rats, like many other animals, seem to know instinctively those who are fond of them and those who are not. This characteristic develops when they are quite young. They learn to distinguish kindness and attention readily, and remember

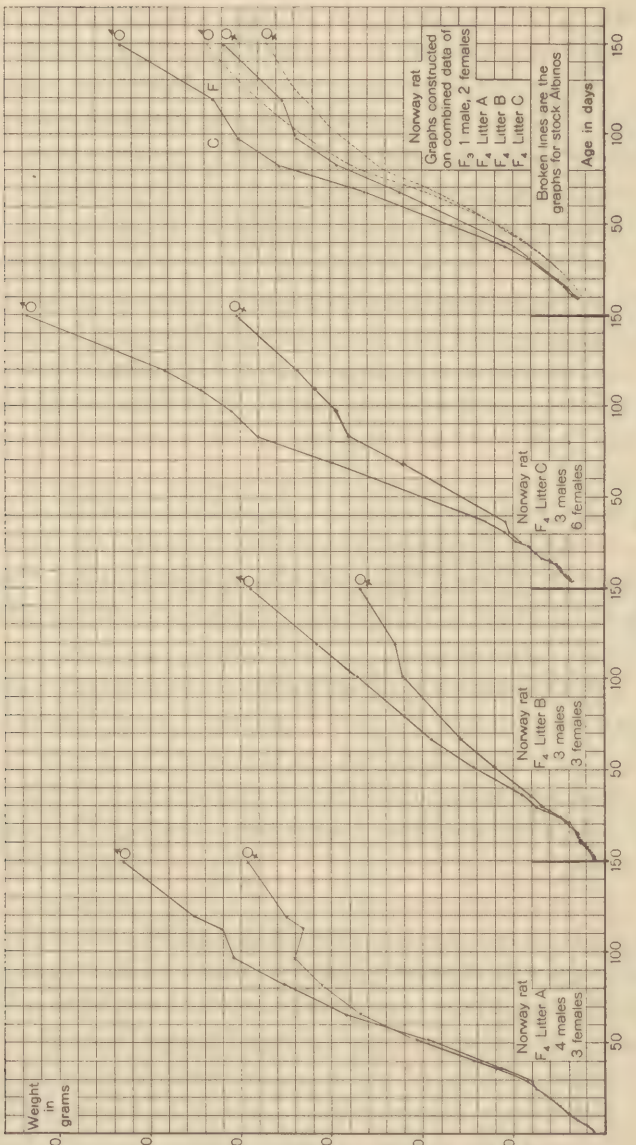

Weight in grams
300
250
200
150
100
50
Norway rat
F4 Litter A
4 males
3 females
Norway rat
F4 Litter B
3 males
3 females
Norway rat
F4 Litter C
3 males
6 females
Norway rat
Graphs constructed on combined data of
F3 1 male, 2 females
F4 Litter A
F4 Litter B
F4 Litter C
Broken lines are the graphs for stock Albinos
Age in days
50
100
150
C
F
♂
♀

Chart no. 2

it easily, and soon expect it when one comes near the cage; therefore, too much care cannot be exercised in selecting the

Chart no. 3

attendants to work among the rats. The worth-while caretaker is the person who will talk to them while working, and occasionally open a cage door, smooth the rats, induce them to romp and play, or pick out a number at a time and place them

on the shoulders and caress them, or place them on a large table, or let them run on a clean floor, and thus get exercise under the most favorable conditions. Individual attention to the animal is an important factor in producing a good, vigorous rat. No person who does not have a liking for animals or who is in the least inclined to be afraid or annoyed by the rats should be employed as a caretaker. They are quick to recognize fear in an attendant and may then be troublesome.

Under favorable conditions, all rats are playful. As very young pups, soon after their eyes are open, they begin to show this spirit of playfulness which continues until about middle life. They will wrestle with each other in groups of two, three, and more. They will jump and frolic, using the front feet to dab playfully at an attendant known to them and one who will play with them. They appear happy and contented and pleased to see their attendants.

If one opens the cage door or comes close to the cage of gentled rats they promptly come forward and look for attention; they will even leave their food to be handled and caressed.

Most albino rats, especially adult rats, are susceptible to the soothing influence of soft, sweet music, especially the higher notes of the violin.

Records of certain types of music produced by stringed instruments reproduced by the phonograph will frequently in a few moments, even at feeding time, cause the rats to become exceedingly quiet and attentive. They seem to listen intently and click their teeth while the music is being played. As soon as the music stops they become active again.

Clicking of the teeth is the rat's method of expressing contentment and happiness. This habit is universally prevalent among albino rats, and is significant of a friendly, pleased frame of mind. This behavior may be compared to the purring of a cat. Rats that are accustomed to being handled and caressed invariably show their satisfaction by making this clicking noise with their teeth. While they are performing in

this manner the vibrissae move rhythmically, the eyes seem to protrude, and if the rat is being held, the whole body can be felt to vibrate. We have observed the same phenomena in cage-bred, tamed gray rats.

Upon close observation the edges of the upper and lower incisor teeth appear to meet and the clicking sound is produced by striking these edges together; sometimes the lower teeth are rotated under the upper ones, the former being well back of the upper ones, and a grinding motion is carried on; then again, the lower teeth are protruded far beyond the upper ones and a clicking noise is made by striking the lower against the upper teeth.

The more albino rats are handled and petted the better they seem to thrive. The daily caressing and, in a general way, treating rats as one would house pets, soon develops in them a confidence in their attendants to such a marked degree that a female rat with a litter of pups will allow those to whom she is accustomed to take the young from her, even to pet her while she is nursing them, or to remove her from the nest.

Sometimes a pregnant female is very nervous, becoming more so as she approaches term. She may resent any attempts to remove her from her cage or even to touch her, and show her resentment by attempting to bite.

In such cases talking quietly to her for a few moments or softly humming a tune without attempting to touch her will sometimes completely change her attitude towards the caretaker so that she will come to the front of the cage clicking her teeth, as an expression of confidence and happiness, and permit one to lift her out of her cage without the slightest objection.

In the cases where litters are weighed every day beginning with the day of birth, the mother, herself always accustomed to being petted, takes it as a matter of course to have her pups removed from under her, and when the weighing is finished and they are returned to her, she, in the same matter-of-course manner, takes them back from the attendant's hand,

arranges them in the nest according to her instincts, and nurses them as though they had not been disturbed.

This is not the case with timid rats. Ungentled rats become seriously disturbed if their new-born pups are molested. They may even kill their young as a result of handling.

A gentled female with a litter will always take her pups from an attendant when they are offered to her until they are about fifteen days old and carry them away, one at a time, in her mouth. At fifteen days of age the eyes open, and after this time the mother seems to consider them old enough to be left to themselves, and while she nurses them and washes them as usual, she is not so jealous of them, and often will not take them from one's hand.

When the pups are born the male usually lives in a nest by himself. In the revolving cage the male lives in one box and the female with her pups lives in the other. This is according to the wish of the mother.

About a day before she casts her litter the female takes all the bedding and builds her nest, leaving the male only a scanty share of the cage comforts in which to live. If, out of pity for him in his bare surroundings, more bedding is placed in his part of the cage, he is seldom allowed to have it, his mate usually carries it to her end of the cage and adds it to her already abundant supply. In building these nests the female rat is a hard worker. Where shavings are used she uses her nose and front feet and shoves the shavings before her until tired, then frequently reverses the method by turning her tail to the nest and kicking backward with the hind feet to pile up the material.

Not infrequently there is an exception to this manner of living in opposite ends of the same cage when a litter is born —an exception worthy of encouragement. The male at such times shows a peculiar sagacity. He will hollow out for himself a nest with an opening leading to the front of the cage, while the female will construct her nest near the rear of the cage to which she will bore a small tunnel along the side and rear walls of the cage.

The male curls up in his nest with his back to the nest where the female and her pups are located. While they probably sleep in this position for mutual warmth, the female and her pups hold the most advantageous position. If the male is carefully removed it will be found that the pups are close to the side of the nest where his body forms a furry warm wall.

Such nests are readily recognized by the two entrance tunnels, one to the female's nest where she cares for her young and the other to the male's bed.

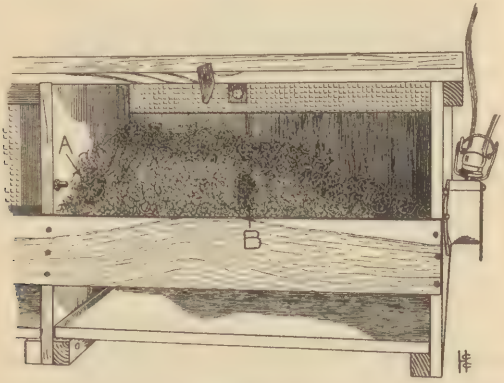


Figure 15

Externally these nests appear as shown in figure 15. In the 'wood wool' bedding may be seen a small tunnel at *B* where the male enters his nest, while at *A* is a more or less concealed opening to a passage along the dividing partition of the cage, then turning along the rear wall to the female's hidden quarters.

A section through one of these maze-like rat homes will reveal a structure which we have attempted to show in figure 16, where at *C* is shown the male's nest entered by a tunnel from the front and adjoining the female's nest *B*, the approach to which is along the side and rear walls of this division of the cage. At *E* the female has still another entrance or exit to the adjoining compartment of the cage without exposing herself near her pups. The drinking fountain is located at *D*.

We try never to disarrange the nest which a pregnant female has prepared for her young. Furthermore, it is inadvisable to change or disturb her nest arrangement for several days following the birth of her litter. Such precautions will relieve the female of any extra worry or work in rebuilding her nest and will react favorably upon the growth of the young.

In the case of gentled females, the disturbance of her nest apparently makes little difference in her welfare.

Instances have been noted where the male is not satisfied just to lie near the nest containing the pups, but hovers the whole litter whenever the female leaves them, and has also been observed to reach over the wall of the nest and pick up one, and sometimes several, of the pups, place them under his body and remain with them for long periods. This latter procedure, if allowed to continue too long, is detrimental to the young, as they are deprived of necessary food.

When the pups are about two weeks old and are able to leave the nest and walk about the cage, they may often be found sleeping on the male's back or on his head. He seems to invite this, for he keeps very quiet if they are playing around him or on his back.

During unusually warm weather, especially when it suddenly follows cool weather when all cages have been heavily bedded, the rats may be seen burrowing under the bedding and lifting it from the wire-cloth floor of the cage and rolling it back so as to expose the cool wire-cloth floor of the cage. Here the mother rat will place her pups without bedding that they may keep cool.

Not infrequently, during such a time, she will separate her pups into two or three groups and nurse each group separately.

If she has access to a revolving cage, she may sleep there in order to keep cool. Then, as if she had warning of an approaching cold wave, the female, just preceding a drop in temperature, will gather all her pups into one group, pull the

nesting materials about them, and cover them with bedding to protect them from the cold.

During cold weather, the female is careful when leaving her pups to cover them with bedding that they may be warm while she is not hovering them.

Mother rats have a means of talking to their offspring just as other animals do, though it is not so easily observed.

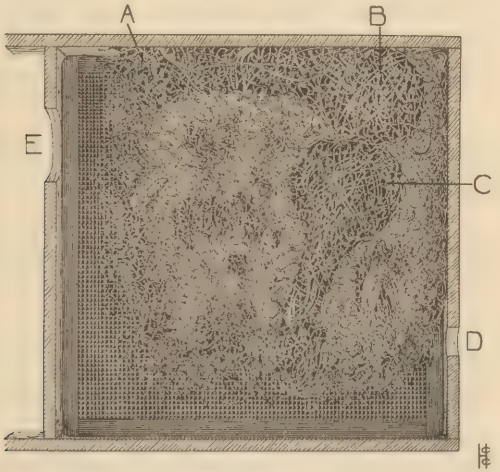


Figure 16

When a mother rat has been away from her nest for some time she gives voice to peculiar reassuring notes as she nestles down among her pups. Occasionally a very gentle mother rat will utter these notes while being caressed. This expression may be compared to that of a mother cat as she gathers her kittens about her.

Anger in the rat is expressed not alone by attempting to bite, but by a peculiar rapid whipping of the tail. Sometimes the entire tail is lashed back and forth and sometimes only the tip end keeps up a continuous undulating motion.

Adult rats require from one to six days to become accustomed to the revolving cage.

Usually one or two days is sufficient for them to learn to operate and enjoy the wheel.

Individual rats differ in their adaptation to this form of exercise. Some find the exercising wheel attractive and are continually operating it, while others take less kindly to it.

The female spends much more time in the revolving cage than the male and takes much more exercise than the male.

It is especially important that the female should have opportunity for exercise. As a result of a highly varied diet, gentling, and exercise, it is not only possible to produce heavier animals, but their fertility also may be greatly increased.

The average number of pups in litters produced by the methods here described is 8.7.

Exercise and food are doubtless the chief factors, while gentling is the necessary preparation which permits them to take full advantage of exercise and food.

A female appears to appreciate the advantages of the exercising wheel. When her pups are able to go out into the wheel, she will operate the wheel while they cling to the wire of the revolving drum.

Occasionally a female will carry her pups from the revolving cage to the nest box. This happens when the pups are from twelve to fifteen days of age. She apparently thinks they are too young for exercise. Later the female will sometimes take one pup at a time in her mouth and run with it in the revolving drum, then return this one to the nest box and take another out for similar treatment until the entire litter has been with her in the revolving wheel.

Adult albino rats live happily in large cage groups if they have been raised together. It is sometimes necessary to place strange rats together in the same cage. In such cases they may not get on well together and are inclined to fight during the first twenty-four hours or until they become accustomed to one another. This is especially true if they have not been gentled. After one or two days they become quite contented with new associates.

When gentled rats are placed together in the same cage they may not fight, but sometimes show their resentment in peculiar ways. For instance, we have observed one rat wall off a new arrival in his cage by building a low wall of bedding across the cage, thus shutting off the intruder. This wall was promptly torn down and as promptly rebuilt. After several days of this performance the two rats became friendly.

BREEDING

In breeding and rearing albino rats for research purposes it should be constantly in mind that the production of a healthy, fertile, normal stock is desired. The ideal stock should attain a body weight and size, including organ weights, of predictable quantities for any age during the life of the rat.

With a complex organism like the albino rat, subject to conditions of food and environment, which can be but imperfectly known and controlled, this ideal can only be approximated. Such approach, however, is very much worth while, since it makes for greater accuracy in any experimental work with this mammal.

Unless there are special reasons for not doing so, it is advisable to inbreed a stock of albino rats intended for research purposes.

Inbreeding, with careful selection of breeders to secure good physical specimens, tends to eliminate the undesirable qualities and to produce a more uniform or homozygous strain.

Brothers and sisters may be mated with no unfavorable results when breeders are carefully selected, as shown by King ('18).

In selecting breeders it is necessary to consider their age, ancestry, and physical condition.

There is a considerable variation in the age at which the albino rat female becomes sexually mature. We have recorded one instance where a female cast a litter at fifty-seven days of age and another instance as early as fifty-five days of age. In our colony, not infrequently females bear litters as early as sixty days of age.

Long and Evans ('22) observed that in 200 rats the average time for the opening of the vagina is on the seventy-second day of life, and the average time for the first ovulation is on the seventy-seventh day of life.

While the average albino rat is sexually mature at about eighty days of age, it is inadvisable to breed them at this age. The first litter of a female bred at eighty days of age or less is likely to be unsatisfactory. The mother may be unable to nurse her young.

It is our practice to delay mating until both sexes are from 110 to 120 days of age. When bred at about this age, the average number of pups in a litter may be expected, and the mother is not likely to fail in supplying ample nourishment during the nursing period.

From 110 days to 350 days of age the female albino rat is in her most vigorous reproductive period. This is assuming that she is not permitted to mate until 110 days of age. After 350 days of age, when she has given birth to five or six litters, the reproductive function declines rapidly until the female is about 450 days of age, when breeding ceases.

Our (unpublished) records show one female who cast her first litter at 77 days of age and her last litter at 581 days of age, producing in that time—less than 17 months—15 litters with a total of 131 pups (average 8.73 young per litter). While such performance cannot be expected in many cases, it may be approached by proper selection of breeders, care, and adequate diet.

In selecting breeders it is well, if possible, to select from a strain which has a record for high fertility and good-sized animals. The presence of these characters indicates a well-nourished strain. Furthermore, it is desirable that the stock from which breeders are selected shall have been reared for one or more generations in an exercising cage during the period of rapid growth, from 25 to 130 days of age. In deciding upon the physical characters which indicate a good breeder, we have made it a practice to select those which dur-

ing the first twenty days after birth appear to be well nourished, short coupled, and broad across the hips. New-born pups show a very considerable difference in the relation of total body length to breadth of hips. As a result of this selection we have a stock which is short for its weight.

When the selected breeders are about 100 days of age they should be examined for any physical defects. A breeder should be plump and well rounded by good muscular development, the skeleton should be well covered. There should be no hollow or lanky areas about the flanks. The animal should have a firm feeling when held in the hand. The head should be symmetrical and well shaped; the nose straight, showing little or no hump between its tip and the bregma. The face should be full and not present a pinched appearance. The hair should be glossy, slightly ivory tinted, and lay smoothly on the body. The teeth should be normal. There should be no signs of lung infection or parasites.

The selection of breeders is an art not easily described, but with a little experience one is soon able to recognize a good normal physique in an albino rat.

Albino rats thus selected for breeders should be from among those gentled by constant handling and petting, if possible, from the time they are born.

If for any special reason it is necessary to breed from a poor stock, it is by no means a hopeless task to rebuild lost vigor. Special care in feeding, with hot broths, hot milk, or hot cocoa, given once or twice daily with other foods, will in many cases restore an ill-nourished or sick rat to good physical condition.

Having selected the breeders, it is advisable to separate the sexes by the time or before they are 35 days of age and to rear them in separate cages until they are 110 to 120 days of age.

Three methods may be practiced in breeding albino rats: First, continuous breeding of one male with one female; second, breeding one male with two or more females; third,

breeding several males with several females. The first method has given us the most satisfactory results and is the method here stressed.

The oestrous cycle in the albino rat or the recurrence of heat or rut takes place about every five days. Little time is therefore lost if breeders are mated at any time.

For more accurate determinations of the occurrence of oestrus, a microscopic examination of the vaginal smear, as indicated by Long and Evans ('22), should be followed.

When one male and one female are caged together at from 110 to 120 days of age, a successful mating will usually take place within three or four days. It is inadvisable to remove the male from the cage when the female becomes pregnant or after her litter is born. He will not destroy the young if conditions are normal.

His instinctive sense of duty towards the pups is very strong, as shown by such instances as where a mother with a young litter having sickened and died the male made every effort to protect the young.

As the female may mate again immediately after the birth of her litter, the presence of the male at all times accelerates the process of reproduction.

The period of gestation is about twenty-two days, with slight variations. In many instances our (unpublished) records show that only twenty-three days elapsed between the birth of one litter and the birth of the succeeding litter, while the mother suckled a litter during that period. Not infrequently we have observed the period between the births of two successive litters to be only twenty-one days, while the mother suckled a litter during that interval. According to our experience, the gestation period is not prolonged by the presence of a suckling litter when proper nutritional and hygienic conditions are maintained at a high level.

It is necessary to remove one litter from the cage just before the succeeding litter is born, as the older litter would continue to nurse while the new-born litter would stand little

chance of getting proper nourishment. Furthermore, the milk secreted immediately following parturition appears to be essential for proper growth of new-born pups. The albino-rat female, like many other mammals, eats the placentae. There is experimental evidence, as shown by Hammett ('18), that placenta fed to a lactating mother stimulates the growth of nursing young.

In continuous breeding with one female and one male in each cage, a litter may be expected about every twenty-five days during the first four or five months of breeding activity. With this in mind, it is advisable to watch for the signs of pregnancy in the lactating female about fifteen days following the casting of her last litter: a little experience will enable one to determine by palpation at this time the presence of a gravid uterus. By such observations and the condition of the female as she approaches term, one may usually predict within a few hours the time for casting her next litter and remove her nursing pups before the new litter arrives.

It is well to give the female at least one full day and preferably two or three days of rest and recuperation from nursing just before casting a litter. During this resting period she should be well cared for and well fed. When the new litter is cast it will get the benefit of the first milk secreted by the mother after its birth. The weaned litter should be specially fed, as indicated elsewhere.

The number of young in a litter varies greatly. Under favorable conditions the average may reach 7 or 8 or even more. The largest litter we have recorded was 18. The average number of pups in the first litters of our experimental series was 9.97, in the fifth litters the average number was 6. The number in a litter depends also upon proper kind and quantity of food and the care bestowed upon the animals. When the albino female is properly nourished and properly cared for, and when she is not permitted to mate until 110 to 120 days of age, her fertility, as expressed by the number of young cast in a litter, is at its maximum in her first litter.

The first litter is the largest in number, and in successive litters the number of young gradually diminishes.

Until the experimentalist has had some experience in breeding and rearing albino rats he may not secure maximum results.

In albino-rat colonies under the usual conditions and with early breeding first litters are not as good as second litters. The average number in a litter is usually 7 or less.

The young of one litter should remain with the mother until just before the next litter is cast, but not longer than thirty days, even if the mother should not become pregnant again.

When first weaned the body of the young rat has a soft, spongy feeling, especially if the litter is a large one or the mother is pregnant while nursing it, but during the first week after weaning, this condition rapidly changes to one in which the body presents a firmer feeling as it increases in weight. This change is especially noticeable if whole milk is fed daily with other foods.

When removed from the mother, the young rats should be caged by litters or in groups of approximately the same age, so that the food may be adjusted to their requirements. If they are to be reared as individual litters, the sexes should be caged separately.

It will be satisfactory for certain types of research to follow the second method mentioned and breed one male with two or more females. This method is satisfactory when a number of pregnant females are desired. As they show pregnancy they may be removed from the mating cage.

For the rapid production of large numbers of albino rats, the third method of breeding may be followed by placing a number of males with a number of females in the same cage. Under these conditions, breeding takes place rapidly and the young animals breed as soon as they are sexually mature. Litters are often pooled and lactating mothers may nurse any pups that desire nourishment.

Many new-born young are destroyed and others poorly nourished by this mixture of all ages, and there is little to recommend the method except the quantity of rats produced. If carried to extremes, as by placing a number of breeding males and females in a small room to run free and breed, the results are not as favorable as when the rats are kept in cages.

Proper hygienic conditions are essential for the production of healthy animals. Cages should be kept clean and dry. The frequency of cage cleaning will depend upon the number of rats in a cage and the kind of food supplied.

We have found that satisfactory conditions may be maintained, where one pair is housed in a dormer cage, by cleaning once each week. This is on condition that a fairly dry food is supplied.

It is advisable to clean the cage of a pair of breeders just before the litter is born and not again for at least one week, and thus avoid disturbing the mother rat while her pups are very young. After a few days she will be more inclined to leave her nest to play and the disturbing effects of cage cleaning will not be so marked.

While it is necessary to clean cages, it should be remembered that the rats dislike to have their nests and hiding places disturbed. Due consideration, therefore, should be given their feelings during the cleaning process.

Early recognition of sex

In most experimental work with albino rats the recognition of sex at birth and later is essential.

Jackson ('12) has pointed out that the most dependable external character by which to recognize sex at birth is the ano-genital distance. This distance he has measured from the anal aperture to the base of the genital papilla (clitoris or penis).

The ano-genital distance is always much greater in the male than in the female of the same age.

This is shown in figure 17, where the distance between points *A* and *B* on the male is greater than the distance between *C* and *D* on the female. Furthermore, the genital papilla of a male is much larger than the genital papilla of a female of the same age. These two characters apply at birth and at all subsequent ages.

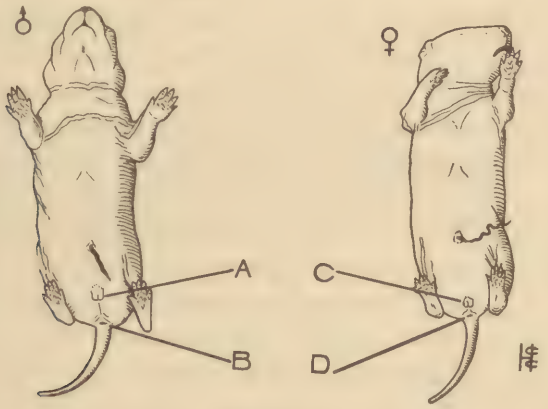


Figure 17

In addition to these external sex characters the following may be noted:

At sixteen or seventeen days of age, when the rat has acquired a coat of hair, the line between the anus and the genital papilla of the female remains relatively bare, while in the male this line or space is covered with hair except a small area just ventral to the anus. This small bare area corresponds to the dorsal part of the scrotal area where, after the sixth week, the testes may occasionally be found.

From eight to fifteen days of age the mammae (nipples) of the female, six pairs, are clearly visible. Later these are obscured by hair.

Birth records—lineage

In all experimental research with a living organism the growth changes which accompany age must receive due con-

sideration. This is especially important in the case of the albino rat during its early life or until 150 days of age. It is usually necessary, therefore, to keep accurate birth records of all litters.

There are also certain physical signs of age which will aid the investigator in verifying the age of albino rats in cases where records or observations are imperfect or lacking.

Addison and How ('21), in a study of the development of the eyelids, have shown that the eyes of the albino-rat embryo are uncovered until early in the seventeenth day, that the lids form rapidly on the seventeenth day, grow across the eye, and their epithelial margins fuse during the eighteenth day of foetal life. They remain closed until the fifteenth day after birth, when the lids separate, beginning approximately in the middle of the eye and opening toward the inner and toward the outer canthus. Usually, the eyes of the females open from twelve to twenty-four hours before those of the males.

We have noted also that not infrequently at the time of the opening of the eyes, the pups show a slight tendency to fail in the usual daily gain in weight, or even to lose weight.

During the period while the lids are joined together, the histogenesis of the retina takes place; when the eyes open on the fifteenth day, all the cell layers of the retina are well defined, though their relative proportions are changed somewhat by later growth.

The external ear, which at birth is folded forward over the external meatus and fused with the skin of the head, unfolds on the third day, with a variation of about one day earlier or one day later.

Addison and Appleton ('15), in their study of "The Structure and Growth of the Incisor Teeth of the Albino Rat," state that from the eighth to the tenth day of age, the incisor teeth erupt, while the second molars appear at about the nineteenth and twenty-first days, respectively. The third molars appear about the thirty-fifth day of age.

There are other physical signs of age, such as the opening of the vagina on the seventy-second day and the descent of the testes about the fortieth day of life. These signs, however, are subject to such wide variation that they cannot be considered of much value in determining age in individual cases.

When the rat is between six and twelve weeks old the new coat of hair develops. The first or puppy coat of white downy fur is being replaced by a new coat, heavier and slightly yellow tinted. The growth of the new coat begins on the ventral surface and sides of the body. It is most noticeable just before it meets along the middle line of the back. At this time the edges of the new and thicker fur coat are separated by a wide space of puppy fur on the head and at the back of the neck and by a narrow strip of puppy fur down the middle of the back which widens again near the root of the tail. The development of the new fur coat is more marked in winter than in other seasons of the year.

It is also desirable and sometimes absolutely necessary to keep records that will enable the investigator to trace the lineage of any individual. For this purpose every mating of one male with one female is numbered and the number of the mating from which the male is derived and that from which the female is derived are recorded.

It is our practice to record all births in the colony at about the same time each day. These records are written on the cage labels. At the same time card records are made for the Colony Office Calendar giving the birth records for the day of all litters and their location in the colony.

The disposition of weaned litters depends upon the investigation for which they are to be used. Both sexes of a litter may be caged together as an individual litter and so recorded. Or the sexes may be caged separately as an individual litter. Or, as is usually done when rats are not used at early ages in individual litters, the excess stock is kept in mixed-litter groups, the sexes being separated.

These groups are composed of rats of approximately the same age and are designated as rats born during a definitely stated time interval as, June 1 to June 15, 1923. The time interval may be longer for older rats, but the age should always be known within one month.

In keeping records of births and ancestry we have found the following record forms, illustrated on pages 85, 87, and 89, of service.

Label no. 1 is used on the cages. Each label bears the number of the cage to which it belongs. If a special series of rats is bred for an investigation, this is usually designated by a number or a letter, as Series A.

The derivation of the male and that of the female is indicated by a mating number. The number of their generation from a given ancestor is indicated as F_1 or F_3, etc.

When a male and a female are caged together, the mating is dated and given a number on the label, the next in the series. Only one series of mating numbers is used for the entire colony. As litters are born they are indicated by letters and their birth days recorded.

In order to record the number of young in a litter and its sex ratio at birth and to have the statistics of the colony in convenient form for reference, we have used record blank no. 1.

On this record blank appear all the data recorded on the cage label; in addition, blank spaces are provided for recording the number of males and the number of females of each litter. The square spaces are used for recording the number of males and the round spaces for recording the number of females.

In order to have a record of the entire population of the colony giving the ages and locations as indicated above, for use in the office of the colony, each birth is recorded on a birth-record card shown on page 87.

Cage	Male	Mated	Female	Litter Born	Litter Born
No. ____	From Mating No. ____	____	From Mating No. ____	A ____	F ____
Series ____	F	Mating	F	B ____	G ____
	Litter ____	No. ____	Litter ____	C ____	H ____
	Born ____		Born ____	D ____	I ____
				E ____	J ____

Label no. 1

	Series	From Mating No. F Litter ___ Born	Mated	From Mating No. F Litter ___ Born								
F	Sex Ratio	○	○	○	○	○	○	○	○	○	○	
	Litter	A	B	C	D	E	F	G	H	I	J	
	Born											

Record blank no. 1

Each card, size 125 x 75 mm., gives the cage number, the strain, and the date of birth of a litter. They are arranged according to the months of the year so that we may locate instantly the litters born in any month. The year is indicated by the color of the card. Three colors are used. The subsequent disposition of litters is recorded from time to time on these cards. By this card record it is easy to locate rats of any age or strain.

When litters have been disposed of as stated above, one of the cage labels shown on page 89 is used.

Timing insemination

For certain researches, especially embryological investigations, it is necessary to obtain the exact time when a female is inseminated.

This may easily be done by selecting young females, from 110 to 120 days of age, and noting by the Long and Evans method what stage of the oestrous cycle they happen to be in or by testing their behavior with a male. Two or three females in heat should be caged with one male.

The most convenient method is to select females of proper ages which show the characteristic swelling of the radiating folds of the vaginal opening, which is noticeably distended at this period, indicating a condition of heat. Such females will usually mate at once.

It is necessary to place the mating cage so that their activities may be observed without disturbing them.

Mating usually takes place more readily in the late afternoon. Several matings should be permitted. These take place usually within a few minutes. One mating of course is quite sufficient if it is completely successful.

During coitus the female throws her head back, her tail forward and arches her back in a concave position. The male may make several unsuccessful attempts at coitus, following which he usually rolls back on his hips and licks his genitals. Following a successful coitus, he usually rises on his hind feet

CAGE NO. _____ Strain_____ **Litter born** _____ **1923**

Litter transferred _____

Single Litter

Both sexes to Cage No._____

Males " " _____

Females " " _____

Mixed Litters

Dates of birth from _____ to _____, inclusive

Males to Cage No._____

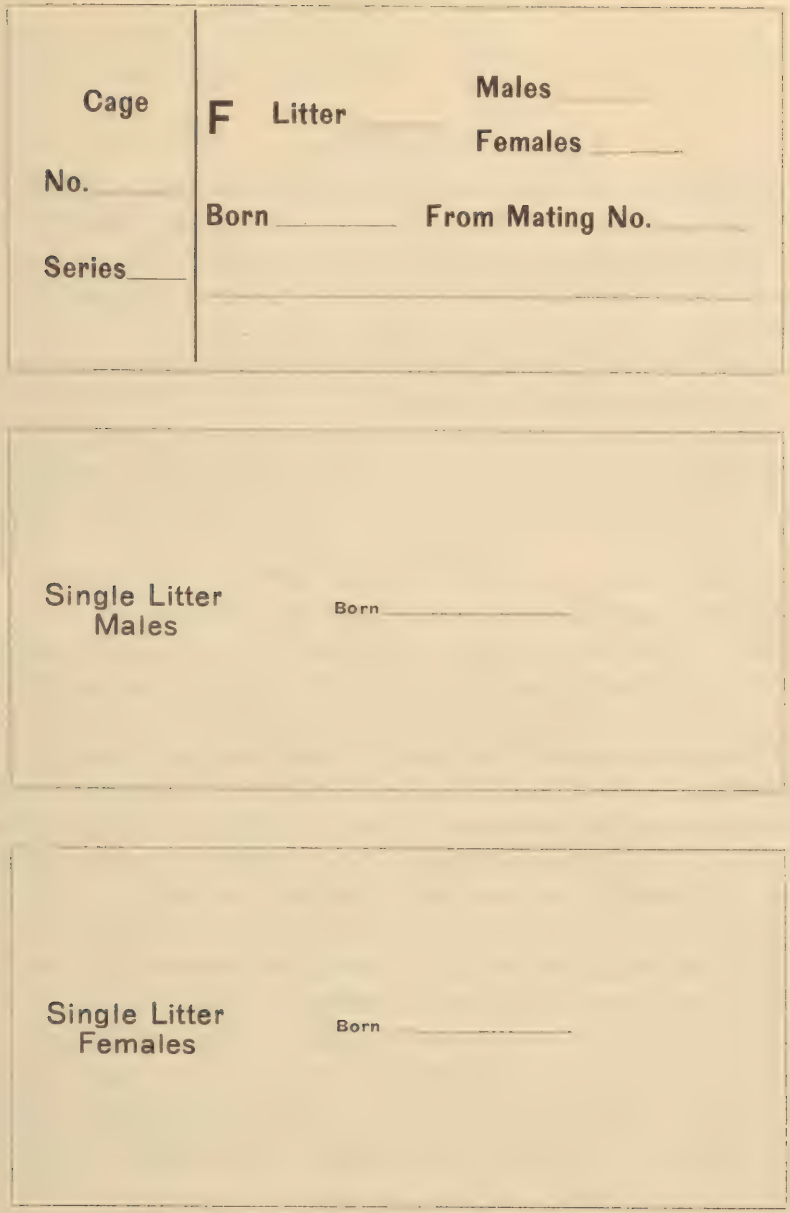
Females " " _____

Birth record card

and makes no immediate further attempt with the female. None of these actions, however, are positive indication of a successful coitus.

Successful coitus may be determined by examining the vagina for the vaginal plug which is always left by the male after successful coitus.

In timing wild gray rats, the mating cage must be in a quiet room by itself and the observer must remain motionless for some time. The least move or noise will distract or frighten the rats. By such methods we have succeeded in timing cage-bred wild gray rats. When the mating process is completed, the females should be caged separately from the males.


Cage
No.
Series
F Litter
Males
Females
Born
From Mating No.
Single Litter
Males
Born
Single Litter
Females
Born

Cage labels

PARASITES

The parasites of the albino rat most frequently found by the breeder are briefly considered here because of their detrimental influence on growth, breeding, and the modification which their presence produces in the normal metabolism of the rat.

Parasites fall naturally into two groups, the internal parasites and the external parasites.

Of the internal parasites it has been our experience that the Cestoda, or tapeworms, are the forms most frequently observed in the albino rat. This may be due to the fact that the encysted larval tapeworm, Cysticercus fasciolaris, a form common to rats, is so easily observed in the liver of the albino rat, appearing there as a small yellowish nodule.

The Nematoda, or true round-worms, and the Acanthocephala, or thorn-headed worms, are also found in the albino rat.

Protozoa are quite frequently, if not always, found in the intestinal tract of the albino rat. Serious infections should be eliminated by treatment or, better still, by destroying the animals. Trypanosoma, found in the blood, render albino rats undesirable for accurate research, although the wild gray rats seem to be but little disturbed by this infection.

The external parasites of the albino rat concern the breeder much more frequently than the internal parasites.

Three groups of ecto-parasites may be found on the albino rat, the Siphonoptera, or fleas, the Anoplura, or lice, and the Acarina, or mites. Occasionally, one of the Cimicidae, Cimex lectularius, or common bedbug, may infest a colony of albino rats.

Fleas (Siphonoptera)

While it has been our good fortune never to have been troubled with fleas in our colony, a word of caution may be in order.

As a number of species of fleas are common to cats, dogs, rabbits, mice, monkeys, rats, and other small rodents, it is essential that a colony of albino rats should be protected against infestation by contact with other animals.

Access of wild gray rats to the colony is especially to be avoided. Not only may the albino-rat colony become infested by such parasites, but it should also be remembered that the flea (Xenopsylla cheopis, Rothschild, 1903) is considered to be the principal means of conveying bubonic-plague infection to man. In seaport cities especially, wild rats may be suspected of bringing parasites bearing plague infection.

Lice (Anoplura)

Lice found on the albino rat are readily recognized by their long, flat bodies, small heads with simple three- to five-jointed antennae and a simple eye on each side of the head. The thorax is incompletely divided into three parts, while the abdomen shows eight segments, six of which have a breathing pore on each side. All segments of the abdomen bear a few simple hairs, the longest on the posterior segments. There are three pairs of stout legs. The color is of a pearly white character. They are parasitic during their entire lives and deposit their eggs on the hairs of their host.

The species most commonly found on the rat, according to Banks, is Polyplax spinulosus, Burmeister, it measures about 1.4 mm. in length.

Mites (Acarina)

Mites are readily distinguished from the fleas and lice by their four pairs of legs, by their oval bodies—not divided into thorax and abdomen—which are covered on the dorsum by a corneous shield bearing stout curved bristles and on the ven-

ter by plates bearing bristles along their margins. Unlike the lice, they are not parasitic during their entire lives and certain forms readily leave their hosts.

The mites which concern the albino-rat breeder most seriously belong to two families, the Gamasidae and the Sarcoptidae.

The Gamasidae, represented as we have usually found them by the genus Laelaps, are visible to the naked eye, measuring from 0.75 to 1 mm. in length, vary in color from a pale pink to a dark red or black, probably depending upon the stage of development.

They are found usually on the back of the albino rat near the root of the tail. They are also found in the litter and on the walls of cages. In the colony they migrate from one cage to another, especially where several cages are carried on the same cage support. They are easily carried from cage to cage by caretakers on their hands and clothing and on implements used in the colony.

The Sarcoptidae are much more minute in size, and in the colony are seldom seen by the naked eye. They are the scab or itch mites which burrow in the skin to live, lay their eggs, and die.

The scab mite found on the rat is whitish in color with an oval body and four pairs of short legs; the two anterior pairs extend beyond the body and end in pedunculated suckers, while the two posterior pairs are not visible from above, and each ends in a long bristle. The integument is semitransparent and shows numerous parallel folds and a few small bristles. The female is much larger than the male.

The species Notoedras muris, Mèguin (Sarcoptes alepsis, Railliet and Lucet) is, we believe, the form found on the albino rat.

Its presence in the skin of the albino rat is indicated by minute reddened and swollen areas varying in size from ½ mm. to 2 mm. in diameter. These areas may be few and scattered or they may be so numerous as to give the skin an exceedingly roughened and inflamed appearance.

Along the tail and on the edges of the ears are the more common areas for the scab mite to penetrate the skin and deposit its eggs as it burrows in. The entire tail may become thickly infested with these burrowing parasites, causing the skin to be exceedingly rough, reddened, and even bleeding. The edges of the ears may also become so infested as to present a very much thickened and an inflamed border.

In bad cases the tip of the nose may also be attacked and a horn-like process of 2 or 3 mm. in height may form on the extreme end of the nose.

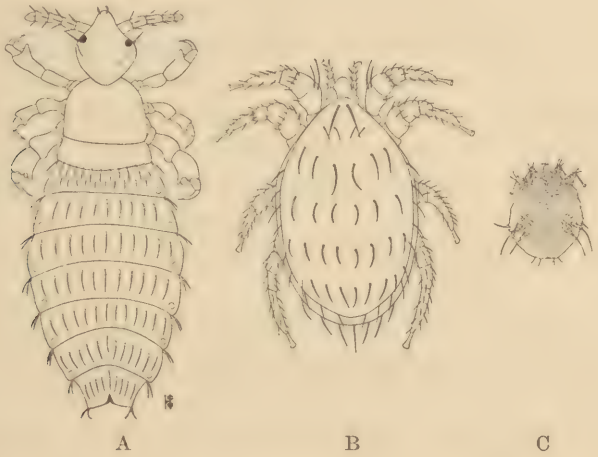


Fig. 18 A, Louse, Polyplax spinulosus, Burmeister; B, Mite, Laelaps echidninus, Berlese; C, Scab mite, Notoedres muris, Mèguin. Showing relative size. × 35.

Prophylaxis—Treatment

Too much stress cannot be laid on the havoc wrought on the general physical welfare of the albino rat by parasites. The effects are shown by the dull and listless reactions of the rat, the pinched expression of its face, and the crouching position in which it sits. The fur is chalky white in appearance and stands out straight from the skin like bristles on a brush. Often the coat looks thin and the skin may even be bare in spots.

The ivory-white tint and smooth glossy sheen of health is lacking. The rat looks anaemic.

Internal parasites. It has been our experience that internal parasites are less of a menace to the albino-rat colony than the external parasites. The life-history of internal parasites may offer an explanation of this apparent difference.

The forms of internal parasites most commonly found, like the tapeworm, depend for their development upon an intermediary host.

As the tapeworm usually found in the albino rat develops to adult form in cats which have fed upon rats carrying larval tapeworms, the eggs of the tapeworm are distributed in the faeces of the cat. Food exposed to contact with cats may thus become infested with tapeworm eggs. These facts have led us to cook or to sterilize all grains or foods that may have been exposed to such infestation or that may be suspected of bearing eggs or larva of internal parasites.

The greatest care should be exercised in the preparation, handling, and storing of food for the albino-rat colony. Not only should it be protected from contact with domestic animals like the cat, but especially from contact with wild rats and mice.

Green foods, which cannot be cooked, should be thoroughly washed with running water.

These simple precautions together with the sterilization of bedding, cages, utensils, etc., habitually practiced, will serve to reduce in number if not wholly eliminate or avoid the more common forms of internal parasites.

External parasites. The most common forms of external parasites which may appear on the albino rat are: 1) Lice (Polyplax spinulosus), which spend their entire lives on the host, suck his blood, and deposit their eggs on his hair; 2) mites (Laelaps echidninus and other species), which feed on the host, deposit their eggs, and develop in the litter of the cage, and, 3) Notoedres muris Mèguin, which burrows into the skin of the host to feed and to deposit its eggs; the young

when hatched leave the host to develop in the litter of the cage and return to the host to complete their life-cycle. The presence of one species does not preclude that of others. In actual experience when one parasite is present others are frequently found.

From these facts it is evident that the successful treatment of an infested rat must consider methods of exterminating the adult parasite and the eggs as well, whether they are found attached to the host or scattered through the bedding of the cage.

When once a colony becomes infested, these parasites multiply with increasing rapidity, until the vitality of the rats is very seriously affected.

Partial or palliative measures are little more than useless when one considers the inconstant results which may follow the utilization for research purposes of an animal whose vitality has been modified by the presence of parasites.

The treatment we have followed is very much the same for all external parasites. The various solutions for dipping infested rats have proved ineffective in our hands.

The most satisfactory method of destroying all traces of external parasites on the rats and in their cages is here described.

Before beginning the process which we speak of as 'delousing,' it is essential to equip a clean room with cages, bedding, feeding dishes, and all utensils required for the care of the rats. All such equipment should be carefully sterilized, preferably in a steam sterilizer at 15 lbs. pressure for from five to ten minutes, depending upon the mass to be heated. Or, if this method is not practicable, the cages may be immersed in a 5 per cent compound cresole solution for one-half hour, then thoroughly rinsed in clean running water, after which they should be dried in the sun and open air until all dampness has disappeared. All dishes and utensils to be used in the room may be treated in like manner. It will be necessary to sterilize the bedding, shavings, excelsior, or paper clip-

pings, either in an autoclave or by dry heating. The former is the better method.

The room selected for the rats should be free from all parasites and protected from wild rats, mice, flies, and other vermin. It should be as far removed as possible from an infested colony. Should it seem best to sterilize the room, as is always the case when a room has contained an infested colony of albino rats or other animals, it may be done as follows: Close all openings, where rats or mice might enter, with cement or other suitable material. With a spray pump thoroughly coat the ceilings, side walls and floors with a 5 per cent compound cresole solution. Be certain that all cracks and corners where insects or their eggs might be lodged are filled with the solution. Repeat this treatment after twenty-four hours. Follow the second spraying, after twenty-four hours, with a copious rinsing with hot water. The room should then be given a coating of whitewash containing 2 per cent crude carbolic acid or compound cresole solution. When thoroughly dry it will be safe for clean animals. Should the infested colony be near the cleansed room, it is advisable to protect the room from crawling insects by a continuous coating 2″ wide and 1/16″ thick of crude vaseline on the door sill, jambs, and head of the door entering the room. Persons passing through the door must step over this strip of vaseline. A mat saturated with 5 per cent compound cresole solution should be kept just outside the door. This mat should be used to wipe from the shoes any eggs or parasites that might possibly be picked up from the floor. In rooms where the compound cresole solution spray and whitewash would do damage to walls or wood work, the same results may be accomplished by using formaldehyde fumigation followed by paint or varnish.

When the room, cages, and equipment are clean and ready for the 'deloused' animals, the workers should be divided into two groups, one working with infested rats, the other taking charge of the cleaned animals.

For use on the infested rats prepare a solution as follows: 1 part tincture of larkspur seed, 3 parts 90 per cent ethyl alco-

hol (measured by volume). Provide an anesthetizing jar with a few shavings in the bottom and a tight-fitting lid. The jar should be of glass in order to observe the exact stage of anesthesia.

A small working table covered with white paper, two or three shallow dishes, preferably white porcelain, a tooth brush, a pair of fine-pointed dissecting forceps, some wiping cloths for drying the rats, and a few ounces of coal oil will be needed. Place the infested rat in the ether jar until slightly anesthetized. The exact stage will be determined by a few tests. The mites will at once come to the surface of the rat's fur and should be brushed into the ether jar or into the coal-oil dish. Lice do not leave the host so readily. Place the rat on its back on the white-paper-covered table and with a tooth brush saturated with the larkspur solution brush the fur under the chin, on the chest, abdomen, front legs and feet, thus soaking the fur on the ventral surface of the fore quarters of the rat while it is completely under ether. Then, holding the rat in the left hand, saturate the dorsal surface and sides of the head and anterior half of the body. Next, place the head of the rat in the left hand, with the third finger firmly pressing against its throat to prevent the rat from biting as it begins to struggle, and, resting its hind quarters on the table, complete the saturation of its fur on the posterior portion of its back and on its hind legs and tail. When the fur of the entire animal has been saturated with larkspur solution it should then be brushed carefully up and down and across the rat's body, including the tail, to make sure that no hair or skin area of the rat has been missed.

All lice, including the nits, and mites, except the itch or scab mites, will be killed at once. The lice and mites should be brushed or picked off the rat with forceps so as not to be mistaken later for living parasites. Nits, which are always attached to hairs, need not be removed, as the eggs are killed by the treatment.

By following this routine the rat can usually be thoroughly cleansed without a second anesthetization. It will, of course,

begin to struggle and is likely to bite any object coming within its reach as it recovers from the ether.

It is advisable to wear a rubber glove on the left hand for this work to protect the hand against the larkspur solution and the bite of the rat.

When very young pups (from birth to fifteen days of age) are treated by this method, it is best to dry them with a soft towel or cloth to prevent chilling, it is possible also that the rubbing aids them, to some extent, to overcome the poisonous effects of the larkspur solution.

This treatment should be thoroughly and quickly applied, several etherizations should be avoided, if possible, the effects on the rat of both ether and larkspur solution are always unfavorable.

After the rat is 'deloused' it should be passed on to the caretaker who is in charge of the clean room prepared to receive the 'deloused' animals. Here the rats should be supplied with sufficient bedding and be protected from draughts, especially in cold weather. It is sometimes advisable to place them temporarily in a drying cage near a steam radiator or in a warm place where they will not suffer from rapid loss of heat.

While the rats appear to be normal after a few hours they do not fully recover from the effects of such treatment for a long time. For this reason, we prefer to use, for research purposes, not the rats which have been subjected to this process, but their offspring of the second and later generations.

As each cage is emptied of its infested rats, its contents should be burned and the cage sterilized.

In handling infested cages with the contained bedding, etc., we have found it desirable to use large pieces of heavy muslin saturated with 5 per cent compound cresole solution, in which cages may be wrapped while transferring them from the colony room to the furnace where their contents may be burned and to the sterilizer where the cage is finally sterilized. Such precautions will avoid the extensive distribution of the parasites and their eggs. Such saturated sheets of muslin spread

upon the floor are often useful and essential when working over infested materials. It aids in preventing the spread of parasites.

If there are indications of the presence of the scab mite, shown by the thickened and inflamed edges of the ears, roughened areas on the tail and about the genitals, a further treatment (see below) of all such rats will be required.

These rats should be separated from those not so affected, isolated in a room by themselves, and preparations made for two or three more treatments. Following each treatment they should be caged in a clean room in sterilized cages.

Cages from which they are removed should be sterilized in the usual manner and the bedding burned, observing every precaution as during the first treatment.

Treatment for scabies

When the rats showing signs of scabies have been 'deloused' by the tincture of larkspur solution and before placing them in the clean cages, they should be dried with a cloth and each little swollen area on the edges of the ears, on the tail, and on other parts of the body should be squeezed with a pair of forceps to force out as many of the scab mites and their eggs as possible. The forceps should be wet with tincture of larkspur solution while doing this and each spot should be thoroughly brushed with the larkspur solution after the squeezing process. All such areas should then be heavily coated with iodex, carbolated vaseline, or balsam of Peru. Any one of these preparations will prevent the escape of the scab mite as it hatches in the skin and attempts to leave the host.

This treatment should be repeated in five days. Two such treatments will usually suffice to exterminate these parasites. The cages should be sterilized between treatments and the bedding burned.

In case of badly infested ears it is more expeditious and quite as effective to trim off the affected edges. The edge involved is usually not more than 2 mm. wide.

No attempt can here be made to consider in detail the diseases of the rat. There are, however, certain pathological conditions occurring frequently in a large colony of albino rats and an occasional instance of defective metabolism that the breeder should be prepared to recognize in order to treat the rats affected or to destroy them as conditions indicate.

Pneumonia

One of the most prevalent diseases, and one which interferes most seriously with research where the albino rat is used, is the so-called 'pneumonia.' Numerous observations have been made on the pneumonias of the albino rat. A number of organisms are always found in the affected parts, but as yet we have no certain knowledge as to what organism is responsible for the chronic pneumonia to which many, especially old rats, succumb. Jones ('22) has succeeded in obtaining from eleven cases organisms resembling those isolated by Theobold Smith from the pneumonic lungs of calves. The calf organism was called by Smith, Bacillus actinoides.

In what we take to be the early symptoms of this infection there is an unnatural, noisy, labored, and quickened breathing. The rat sits with its back humped into an arched position, its eyes are dull and paler in color; the ears and nose are whitish and waxy looking as if the rat were anaemic. The rat feels cold to touch, that is, it does not radiate heat as normally. The fur looks stiff and wiry and has lost its silky, yellowish-white appearance. The rat refuses to eat. The nostrils are reddish, showing a mucous discharge; the eyes are sometimes matted with a reddish discharge. Distinct râles may be heard in the lungs. Following the acute stages, the rat may live for some weeks in a much depressed condition of health, then finally succumb to the disease.

When rats die of this infection the lungs show abscesses of varying extent, from a small mass to the almost complete obliteration of the lung. In its early stages this infection appears to respond to treatment.

The disease appears to begin with an intense inflammation of the entire respiratory tract, including the nose and throat. The animal holds its lips slightly open and is apparently unable to swallow when it puts its head down in the usual position of feeding.

Acting upon the supposition that the rat cannot distinguish food in the usual manner by the sense of smell, is unable to swallow when thus attacked, and gradually succumbs to the infection owing to lack of nourishment, we have treated such rats in the following manner:

Hot milk, hot beef broth, and hot cocoa were all tried as a food for rats suffering from this infection. Hot cocoa proved to be the most acceptable food. This is prepared by heating a small quantity of whole milk and adding cocoa and sugar in equal proportions until a mixture is made of about the strength used on the table.

This is fed every hour to the affected rat by holding its head well back and, by means of a pipette, placing a few drops at a time well back in the rat's mouth and to the lateral side of its teeth. In a few seconds the rat begins to swallow when the cocoa is placed in the mouth. After a few feedings of this kind, the rat will usually take the pipette in its front paws and hold it while the cocoa is forced into its mouth.

After some days of this forced feeding of hot cocoa or other liquid diet, the rats usually show marked improvement and symptoms of the disease subside, abnormal breathing being the last symptom to disappear. When such treatment is begun in the early stages of the infection, recovery is usually rapid, taking place within one week. If treatment is delayed, recovery is much more prolonged.

Unless there are reasons for preserving certain animals of the colony, it is much more economical to destroy all rats which show unmistakable signs of this so-called pneumonia.

Middle-ear disease

Another infection which occurs quite frequently in the albino rat colony is a disease of the middle ear resulting frequently in mastoid abscess. The auditory bulla may become filled with a turbid fluid or pus while the rat shows no symptoms by which its condition may be recognized in the early stages.

The first symptom usually observed is the tendency which the rat shows to carry its head turned slightly to one side. When such a rat is held up by the tail it will usually spin around and around. This indication of a disequilibrated condition is usually accompanied by middle-ear disease which later develops in the form of a mastoid abscess.

Affections of the middle ear are shown by Stotsenburg ('23) to be accompanied by a slight lowering in the weight of the ovaries. It may be assumed that the entire organism is more or less affected by this condition.

It is advisable, therefore, to eliminate all albino rats which show signs of middle-ear disease, since they are unfit for research where a normal body chemistry must be had.

Deficiency diseases

Concerning deficiency diseases a word of caution may be helpful. In 1897 Eijkman in a study of beriberi demonstrated that this disease was due to the long-continued use of polished rice as a staple food. He was able to produce it experimentally in fowls and cure it by feeding the rice polishings which were usually discarded. In 1912 Casimir Funk applied the word vitamines to food factors which are essential in preventing certain diseases.

During the past ten years the number of investigators who have worked on these elusive bodies have increased greatly and the science of nutrition has advanced along new and intensely interesting lines.

Proteins, fats, carbohydrates, mineral salts, and water in adequate amounts are no longer believed to be the only sub-

stances required to promote perfect metabolism. A number of so-called vitamines are essential factors in this process.

The vitamines have not been isolated as chemically pure substances, but their existence is evidenced by lack of growth or by the so-called deficiency diseases which result from their absence. At least three forms are now recognized and usually indicated as vitamines A, B, and C. It seems probable that the list of such essential food factors may yet be considerably augmented.

A lack of one or more of these food factors may result in a failure of the young to grow or in one of the deficiency diseases or in both.

Insufficient vitamine A may result in defective growth and a swollen and inflamed condition of the eyes of the albino rat resembling xeropthalmia. Lack of vitamine B may result in defective growth and a condition suggesting polyneuritis or beriberi. Lack of vitamine C may permit scurvy to develop in the guinea-pig. The rat is said not to be subject to scurvy.

Evans and Bishop report a dietary factor which they designate as X and without which albino rats become sterile. This element is supplied in the fresh green leaves of lettuce.

Just what diseases are to be included in the list of deficiency diseases is yet a matter of discussion.

In maintaining a colony of albino rats it is well to bear in mind that excessive heat and oxidation may destroy or damage some of the essential elements in a food. Also, that food factors not supplied from one source may easily be supplied from another. Cooking should be done with due caution and the food should be varied daily, as indicated in the chapter on Food and Feeding.

We have on several occasions discovered albino rats in the colony showing on the body surface small scabs or areas covered with dried blood. This trouble rapidly disappeared when oranges were supplied in the diet.

Deficiency diseases will not seriously trouble the breeder if due care is given to the question of varied foods.

Faulty dentition

It occasionally happens that through some aberrant metabolic process, the incisor teeth of either the upper or lower jaw or of both jaws become abnormally elongated. This excessive and rapid growth of the incisors interferes with normal feeding. They may even grow to such an extent as to pierce the lips or cheeks or enter the muscle within the zygomatic arch.

The remedy which we have followed, when the rats must be preserved, is to clip off the excessive growth with a pair of bone forceps, leaving the incisor of normal length.

It may be necessary to repeat this treatment from time to time. The growth is usually not so rapid after the first treatment.

It is our belief that this excessive growth is due to a defective diet rather than to the physical condition of the food supplied to the rats. When a highly varied diet is supplied, the cases of faulty dentition are less in number.

For reasons elsewhere stated, it is not possible to maintain a strain of albino rats which will always agree with previously established standards of weight and dimensions for given ages or which will show organs of the same relative weights.

For this reason any colony of albino rats, used for researches of a quantitative character, should be tested periodically to determine the direction and the amount of deviation from previously established values or standards.

The reference values or standards which have been prepared for this purpose appear in a book by Dr. H. H. Donaldson entitled "The Rat," published as Memoir No. 6 of The Wistar Institute, 1915. In this book reference tables for the weight and dimensions of the entire body and the weights of the organs from birth to maturity are presented. From the tables the form of the growth curves for the body and its parts can be derived. The form is largely independent of the absolute weight of the part measured.

In order to fully compare the results of two researches, made for the same purpose, it is necessary to know the physical characters of the groups of rats used on each occasion.

By examining the control rats, the amount of their deviation from the reference tables may be readily determined and thus establish the relative status of any series of rats.

The information thus obtained makes possible a more adequate interpretation of results recorded at different times and in different laboratories than would otherwise be possible, and brings into view the possible influence of variation in the weights of the several organs on the responses of the animal.

TRANSPORTATION OF LIVING RATS

We are called upon constantly to send living rats to all sections of the United States and occasionally to send them beyond our national boundaries.

Our experience may perhaps be of service to someone, hence these notes on transportation.

In boxing rats for shipment one-half of the top and one-half of one side of the box should be open and covered only with wire cloth. This will insure proper ventilation. Boxes should be filled with excelsior so that the rats may burrow and protect themselves from cold. Dry food should be placed in the box and one or more heads of lettuce should be wired inside to the top of the box. The amount of each should be determined by the time the rats are to be confined and the number in the box. It is our practice to use boxes 24″ long, 10″ wide, and 10″ high. Such a box will accommodate twenty rats for a twenty-four-hour trip. For longer trips fewer rats should be placed in a box.

In sending rats long distances it is necessary to partition off a small part of each box, provide a lid which may be opened and fill this compartment with dry food. A door opening into the section containing the rats should be provided and a watering dish should be placed in the box with the rats. Arrangements may be made with the express company to feed and water the rats en route.

In shipping by steamer, similar boxing is required and arrangements may be made with the proper officer of the ship to feed and water the rats.

Pregnant females cannot be shipped before the tenth day following insemination. Resorption takes place at any earlier time and even at the tenth day a very considerable disturbance may result.

We have attempted to carry rats in the earlier stages of pregnancy in a hand bag on a 300-mile trip and failed to get them to their destination without resorption. Any accurate embryological studies should not be attempted on embryos transported for any great distance.

When albino rats have been transferred from one locality to another, even within the same building, they should not be used for research purposes until they have become accustomed to their new quarters and their normal metabolism has been reestablished.

The time required to reestablish normal physiological processes will, of course, depend upon the intensity of the disturbance to which they have been subjected.

Rats which have been removed from an accustomed environment should be placed in dormer cages, fed, watered, and gentled as we have indicated until they have recovered from the fear evinced when placed in new surroundings, and until they eat normally and react as usual to the new environment. Loss of weight may always be expected when albino rats are transferred from one locality to another and placed under the care of new attendants.

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THE WISTAR INSTITUTE COLONY BUILDING

Constructed of brick, concrete, steel, and glass; isolated from all other buildings.